Molecular Detection the Agent that Causing Vaginitis in Vaginal Secretion from Women with Vaginitis and it Relation with Abortion

Aliaa Salman Mahdey[a], **Frial Gemeel Abd**[b]

[a]*Al Qasim Green University, Iraq*

[b]*College of Science, University of Babylon, Iraq*

aaliaa308@yahoo.com frialabd@yahoo.com

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Abstract

One hundred (100) swabs were collected from women with vaginitis ,from the hospital of Babylon city and private clinics. All the samples were taken from married women from pregnancy. The age of patient (17– 40) years. The sample was collected by disposable swabs .These swabs stored in freezing until were used, DNA was extracted from these swabs. The DNA genomic standard is prepared for the 16 S r RNA gene after the using of nanodrop spectrophotometer (ng / ml) for detection agent causing vaginitis by polymerase chain reaction technique .The result appeared that *Candida albicans* was more common in 79.17% while *Trichomonas* in 22.92%, other causes were bacterial vaginosis. Also study the role of the infection agent with abortion, the result appeared that *Megasphaera sp* was high risk with abortion.

Keywords: Bacterial vaginosis, qPCR, abortion

Introduction

Doderlein in 1894 discover (BV) after along study in another studies of sepsis caused abortion or caesarean section [1]. In 1955 (BV) was discovered as a vaginitis and specific pathogen like *Tricohmonas Vaginitis* and *Candida albicans* was not specific to caused vaginitis Gardener and Dukes in 2011 isolate *Haemophilusvaginalis* that non specific for vaginitis [2]. The culture technique for anaerobic gram-negative bacilli, gram-positive Cocci and *Mycoplasma* are considered assign for *Vaginalis*. In 1983 the scientist considered (BV) as a symposium for the anaerobic or facultative bacteria with no inflammation and the anaerobic and aerobic are discussed the overgrowth of aerobic bacteria such *E. coli*, Group B *Streptococci*, *S. aureus* are considered a pathogen state because of *Lactobacillus* is reduced [3]. Vaginal complaints and abnormal vaginal discharge and odor are mainly caused by *Bacterial vaginosis* and the polymicrobia growth due to the inflammation in uterus and Fallopian timization molecular technique that is used for detection very specific. Specific until know is not identified. In China, there is a report about Chinese women that study the relationship between pathogenic bacteria and (BV) and linked with vaginal pH and Nugent scores and abortion [4]. Many studies have shown the use of (q PCR) or semi quantitative (PCR) and until known there is no united procedure Assay for (BV) detection [5]. There's many report around the world about using (q PCR) as a detection [6] all of their research have been used the (qPCR) as assay by using (absence/ presence) as a qualitative detection [1]. In reproductive age women infection vaginitis represented 90% of all cases of vaginitis that composed bacterial infection, candidiasis and trichomonias that have a relationship with opportunistic pathogen [7][8]. Atrophic vaginitis that exist in postmenopausal or post partum women and occur in young girls because the estrogen is poor in concentration and this phenomenon considered[9]. The relationship between the bacterial infection and estrogen concentration is complex [10].

There's some bad habits include hot moist clothes wearing that stimulate growth of pathogens and the dialing of beneficial bacteria that caused by sprays, douches, irritation to epithelial cells and safe sex to avoid sexually transmitted disease especially the women have more ability of infected with sexual transmitted disease [11]. Estrogen have a strong effect on vaginal pH and have a good role to increase redox potential that have less optimize condition for anaerobic organism, and secondly enhance the adherence to epithelial cell[12]. Increasing in Lactobacillusspecies is a result of estrogen used with decreasing of pathogen bacteria especially in p*ostme*nopausal women with vaginitis [13]. Estrogen high concentration due to glycogen concentrati*on in vagina*l epithelial cell because the Lacto-bacilli of the metabolism that the glucose that considered the essential nutrition factor that convert to lacti*c acid* that lowering the vaginal pH [14]. Glucose provide the growth of other organisms from glycogen metabolism and this is available from hormonal *replac*ement therapy and c*onfirm* by [15][16] found this situation as physiological condition and normal. Bacterial vagnosis is a good sign for sexual transmitted disease it caused elevated vaginal pH and alter the host defense mechanisms. If patient, increase the chance of STI it will beco*me mo*re chance to be HIV positive [17], and the main reason for obstetric outcome over the world and pregnancy loss, preterm birth, preterm labour, premature rupture of membrane, amniotic fluid infection post-partumen dometritis and post seaseran wound infection and make ascension through the vagi*na.*[18][19].

1. Materials and Methods

1.1. Patients

One hundred (100) swabs were collected from women with vaginitis ,from the hospital of Babylon city and private clinics. All the samples were taken from married women from pregnancy. The age of patient (17–40) years. The sample was collected by disposable swabs. These swabs stored in freezing until were used

1.2. Molecular methods

According to Zozaya–Minchiff *et al* , 2010 the copy number is detected for Bacterial Vagnosis By using commercial genomic DNA extraction kit by the following steps by using 1.2 ml of microcentrifuge tubes a 300 ml of the media that include vaginal swabs was transferred .The tubes was put on centrifuge for 1 minutes and taken the supernatant . By adding (20 ug/ ml) of 200 ml lysozyme buffer and mixed by vortex. By adding 300 ul cell lysis to every tube by using vortex mixture By incubation at 60c for 10 minutes to ensure the clearness of sample. but the sample should be inverted every 3 minutes. The sample should be incubated on ice for 5 minutes after adding. the 100ul protein removal buffer to the lysates samples. The tubes should be put in centrifuge for 3 minutes at 10000 rpm. After the supernatant were transferred to the tube of centrifuge then the adding of 300 of isopropanol and mixed and mixed. After 5 minutes at 10000 rpm the tube was placed. After the supernatant were discarded 70% ethanol of 300 ul was added to wash the DNA pellet. The tubes was putting in the centrifuge for 3 minutes at 10000 rpm. For 10 minutes and after letting to air – dry the supernatant were discarded. 50 ul elution butter was adding to the DNA pellet for 30 minutes at 60 c and the sample storage at deep Freezing until use.

By using nano dropspectrophotometer the extracted DNA is checked to ensure the purity of DNA by the absorbance of sample at 260/280 nm by the following steps: The nucleic acid DNA was taken to check . The 1 ml of deionized water is added after the cleaning of pedestals with a dry chem.-wipe and added on the lower surface of measurement pedestal. Lowering the sampling arm and the ok was kicked to begin the nanodrop. DNA sample was putting for the measurement. The PCR is used for the identification of the copy number of (BV). The DNA genomic standard is prepared for the 16 Sr RNA gene after the using of nanodrop spectrophotometer (ng/ml).The thermocycler condition for the detection of DNA in the initial denaturation at 95 c for 3 min for 1 repeat cycle and the denaturation at 95 c for 10 sec for 45 repeat cycle and the annealing step in 55 c for 30 sec for 45 repeat cycle and the melting at 95 c for 5 min in 1 repeat cycle.

2. Results

Results related to micro-organism infectious load were presented in two ways; the first one was in the form copy that was obtained from the real time PCR procedure. The second way is a modification of the former results by converting any zero copy number into negative results, by treating any copy number exceeding zero as positive. Accordingly the results were as follows:

1. *Candida albicans* was the major infectious micro-organisms, accounted for 76 (79.17%), and the median copy number was 3.46 while the mean was 3.94+0.3.
2. *Atobopium* sp. came in the second rank and accounted for 67 (69.79%). Median copy number was 3.85 and mean copy number was 4.25+0.38.
3. *Gardenella vaginalis* came in the third rank and accounted for 64 (66.67%). Median copy number was 2.43 and mean copy number was 3.31+0.32.
4. *Lactobacillus acidophilus* came in the fourth rank and accounted for 56 (58.33%). Median copy number was 2.33 and mean copy number was 2.68+0.29.
5. *Bacteroides sp.* came in the fifth rank and accounted for 51 (53.13%). Median copy number was 1.62 and mean copy number was 2.42+0.27.
6. *Mobiluncus* sp. came in the sixth rank and accounted for 34 (35.42%). Median copy number was 0 and mean copy number was 1.63+0.26.
7. *Megasphaera* sp. came in the seventh rank and accounted for 32 (33.33%). Median copy number was 0 and mean copy number was 1.47+0.25.
8. *Mycoplasma hominis*. came in the eighth rank and accounted for 31 (32.29%). Median copy number was 0 and mean copy number was 1.27+0.22.
9. *Trichomonas vaginalis* came in the ninth rank and accounted for 31 (32.29%). Median copy number was 0 and mean copy number was 1.05+0.23.

Table (1)molecular detection of microorganisms that causing vaginitis by Polymerase chain reaction

	Positive		Negative		Copy number	
Micro-organism	**No.**	**%**	**No.**	**%**	**Mean ±SE**	**Median(range)**
Candida albicans	76	79.17	20	20.83	3.94±0.30	3.46 (0-9.68)
Atobopium sp.	67	69.79	29	30.21	4.25±0.38	3.85 (0-9.88)
Gardenella vaginalis	64	66.67	32	33.33	3.31±0.32	2.43 (0-9.43)
Lactobacillus acidophilus	56	58.33	40	41.67	2.68±0.29	2.33 (0-9.24)
Bacteroides sp.	51	53.13	45	46.88	2.42±0.27	1.62 (0-8.36)
Mobiluncus sp.	34	35.42	62	64.58	1.63±0.26	0.00 (0-9.99)
Megasphaera sp.	32	33.33	64	66.67	1.47±0.25	0.00 (0-9.43)
Mycoplasma hominis	31	32.29	65	67.71	1.27±0.22	0.00 (0-8.73)
Trichomonas vaginalis	22	22.92	74	77.08	1.05±0.23	0.00 (0-8.90)

Comparison of infection rate between women with multiple abortions (2 and 3) with that of women with single abortion revealed the following results: Candida albicans: The rate was 76.92% versus 80.7%. The p-value was not significant (P>0.05). *Atobopium* sp.: The rate was 82.05 % versus 61.4 %. The p-value was significant (P<0.05). *Gardenella vaginalis:* The rate was 74.36 % versus 61.4%. The p-value was not significant (P>0.05).*Lactobacillus acidophilus*; the rate was 74.36 % versus 61.4%. The p-value was not significant (P>0.05).*Bacteroides sp.*: the rate was 58.97 % versus 49.12%. The p-value was not significant (P>0.05). *Mobiluncus* sp.: the rate was 35.9 % versus 35.09%. The p-value was not significant (P>0.05).*Megasphaera* sp.: the rate was 56.41 % versus 16.54%. The p-value was significant (P<0.05). *Mycoplasma hominis:* the rate was 33.33 % versus 31.58%. The p-value was not significant (P>0.05).*Trichomonas vaginalis*: the rate was 35.64 % versus 21.05%. The p-value was not significant (P>0.05).In conclusion only *Ato- bopium* sp. and *Megasphaera* sp. were significantly associated with multiple abortions. These results are clarified in table 2.

Table (2)Association between abortion and type of infectious micro-organism that causing vaginitis in women

	Single abortion		Multiple abortions			
Micro-organism	**No.**	**%**	**No.**	**%**	**P-value**	**Significance**
Candida albicans	46	80.70	30	76.92	0.654	Not
Atobopium sp.	35	**61.40**	32	**82.05**	0.030	Significant
Gardenella vaginalis	35	61.40	29	74.36	0.186	Not
Lactobacillus acidophilus	31	54.39	25	64.10	0.343	Not
Bacteroides sp.	28	49.12	23	58.97	0.342	Not
Mobiluncus sp.	20	35.09	14	35.90	0.935	Not
Megasphaera sp.	10	**17.54**	22	**56.41**	<0.001	Significant
Mycoplasma hominis	18	31.58	13	33.33	0.857	Not
Trichomonas vaginalis	12	21.05	10	25.64	0.599	Not
Total	57	100.00	39	100.00		

In order to study the amount of risk of abortion caused by those bacteria, two statistical tools have chosen. These are Odd ratio and etiologic fraction. Women with *Megasphaera* sp. infection had a chance of getting multiple abortion of approximately 6 times than those without such infection (odd ratio =6.082; 95% CI ranged from 2.398-15.429). The etiologic fraction (EF) submitted by *Megasphaera* sp. infection was estimated to be 0.574 which is substantially high. These results are demonstrated in table 3.

Table(3) amount of risk experienced by *Megasphaera* sp.

Megasphaera sp.	Multiple abortion		Single abortion		P-value	Odd ratio	95% CI	EF
	No.	%	No.	%				
Positive	22	56.41	10	17.54	<0.001	6.082	2.398-15.429	0.574
Negative	17	43.59	47	82.46				
Total	39	100.00	57	100.00				

On the other hand, women with *Atobopium* sp. infection have a chance of getting multiple abortion of approximately 3 times than those without such infection (odd ratio =2.873; 95% CI ranged from 1.082-7.628). The etiologic fraction (EF) submitted by *Atobopium* sp. infection has estimated to be 0.311 which is substantially high. These results are demonstrated in table 4.

Severity of abortion has expressed in terms of mean and median copy number. *Atobopium* sp. and *Megasphaera* sp. were the only two micro-organisms that showed significant association with abortion, in such a way that women with multiple abortions had more severe infection than women with single abortion; the p-values were 0.008 and 0.018 respectively.

Table (4) Mean and median copy number of micro-organisms in multiple abortions versus single abortion

	Multiple abortion			Single abortion			
Micro-organism	**Median**	**Mean**	**SE**	**Median**	**Mean**	**SE**	**P-value**
Candida albicans	3.45	3.76	0.46	3.46	4.07	0.39	0.700
Atobopium sp.	5.47	5.08	0.48	2.17	3.04	0.55	**0.008**
Gardenella vaginalis	0.00	0.73	0.29	0.00	0.68	0.35	0.903
Lactobacillus acidophilus	2.65	3.05	0.48	1.92	2.43	0.35	0.289
Bacteroides sp.	2.53	2.81	0.44	0.00	2.15	0.35	0.231
Mobiluncus sp.	0.00	1.80	0.46	0.00	1.52	0.31	0.844
Megasphaera sp.	3.31	2.13	0.32	0.00	1.79	0.36	**0.018**
Mycoplasma hominis	0.00	1.23	0.33	0.00	1.30	0.30	0.968
Trichomonas vaginalis	0.00	1.05	0.34	0.00	1.06	0.31	0.700

3. Discussion

4.1 Bacterial vaginosis and abortion

Bacteria in present studies concert on abortion with BV records *G. vaginalis* is the most dominance with 63.26%, *Megashaera* type 59.18% and *Atomobium vaginae*53.06% and this is confirm with other study and their studies considered *G. vaginalis* as the basic causative agent of abortion [2][20][21][22] [23]. The most characteristic of (BV) that it have no signs and lead abortion and this confirm with [24][25] [26].

In spite *G. vaginalis* is considered as a pathogenic microbes and caused abortion and (BV) but in case there is no harmful effect in woman and this statement confirm with [27] [28]. Present study showed *G. vaginalis* with 66.6% percentage *A .vagina* in our studies showed 69.7% while in other studies showed 53% in previously studies that mention by [29]. *A. vagina* occurs in the absence of *G. vaginalis* so rarely [30]and mention about the synergism between the two pathogen the mechanism that mention that *Atopbium* consumed peptides peptidase and producing memorial in surrounding where the sugar is the main source of energy [31]. *A. vagina* is considered to be specific for (BV) [17][32] this type of bacteria found in healthy women but in low frequency in recent studies [33][34], otherwise in other studies it consider a cause of abortion [35][5]. *Megasphera* 73.7% for both an *G. vaginalis* with 50% concentration and this result was detected by [36][5][37]. In other studies *Megasphera* is considered the main cause for (BV) and the main cause for abortion [24][5][38][39] and it diagnosis in 50% of (BV) [40][41] found in his studies a good relationship between the abortion and the bacterial vagnosis and discovered that women with first trimester in Belgium have a confidence with 95%**.**

With abortion especially *Mycoplasma hominis* and *Ureaplasm ureticum* and his conclusion based on early pregnancy and abortion related with bacterial vagnosis with unclear confirm mechanism. In the present study the odd ratio for *Megasphera* was 6.082 and *Atobopium* 2.873 and with bacterial vagnosis with non-specific kind species for the preterm delivery 2.19 and raised until 4.20 odd ratio and showed the high risk for preterm birth. Current studies shows that with increasing of number of abortion. The percentage of abortion that reach 59.38% as single abortion but current study show that *Megasphera* is high in spit it present is lower from *Atobopium* but odd ratio reach to 6.082 with 85%. Compare with Gillbert that found *Mycoplasma hominis* is more risky in early abortion in pregnant women with high odd ratio reach to 4 more risky from other kind of bacterial vagonsis. This study showed that the multiple abortion be significant and more highly from single abortion with 82.05% percentage them 61.40 percentage that are significant followed by *Megasphaera* with 56.41 , 17.54 respectively with significant value and p value .The studies with the statistical analysis was significant for *Atobopium spp* and *Megasphaera* spp with 0.008, 0.018 respectively. The study showed that *C. albicans* is the most prevalence as a normal flora with pathogen effect with a percentage 79.171 followed by *Atobopium spp* with percentage 69.79% and *Gardenerlla* 66.67%. This result obtained from qPCR and the main caused for (BV) when it isolated from 96 women with and without (BV) with 58.33% *l. acidophilus* 16srRNA gene copy but this studies showed a high risk of abortion with *Atobopium* and *Megasphera* with a high risk of abortion and this result confirmed with the report of [24] [42] [43]. And studies showed that the absent of *L. acidophlus* not means unhealthy condition of women [44].Estimative of DNA extraction by using nanodrop spectrophotometer was measured at (260-280) nm with purity from 1.52 to 2.11 based on the concentration of DNA for PCR amplification (Applied Biosystems, 2008) The PCR showed a big novel of uncultured bacteria from the vagina secretion from women that have (BV) and prevalence (BV) this detection is very important to marker diagnosis and have very important effect as pathogen because of it virulence factor in spite of their low concentration and low abun- dances this study was very reliable for diagnosis and indicators for uncultured bacteria this studies agreed with many studies for (BV) detection by using (PCR) [24] [5] [45] [46] [47] and all of these research used PCR as detection applied biosystems mention in 2008 that the standard curve is a one common strategy in quantification by using the standard curve for extrapolating threshold cycle for extrapolating with unknown concentration for sample and by applied the dilation series for the standard curve for the reference**.** in spite of the differences was significant with the *Lactobacillus* strain and with the 4 group of clean vagina that used as a comparison and the result showed that the normal microbe was replacement with the pathogen that change the balance of the vagina ecosystems and the result showed that the prevalence of some pathogen caused abortion with the records and this result agreed with [48]. The studies based on quantitative PCR for 16s rRNA gene for the (BV) as a tar- get with sensitive and specific diagnosis [49] The quantitative reverse transcription real time PCR depending on syper green master mix for the PCR detection

that depending mRNA transcript level the DNA copy number in ribosomal RNA for the target gene for microbial vaginitis for the causative agent depending on the data of exponential phase of syper green that react with DNA complementary and by application showed different values for the number of threshold cycle and the ribosomal of threshold cycle number that collected for the RNA gene that using amplification plot and genomic DNA as standard curve that used in quantification of the level of mRNA transcript .

CONFLICT OF INTERESTS.
There are non-conflicts of interest.

References

[1] P. R. Mason, D. Katzenstein, R.H.K. Chebira, and L. Mhmaraly, "Puerperal sepsis group, vaginal flora of women admitted to hospital with signs of sepsis following normal delivery, caesarean section or abortion center," *Afr. J. Med*, vol. 35, pp. 344–351, 2011.

[2] J.R. Greenwood and M. J. Pickett, "Transfer of Haemophilus vaginalis Gardner and Dukes to a new genus , Gardnerella," *Int J Syst Bacteriol*, vol. 30, pp. 170–178, 1980.

[3] G. Donders, A. Vereeckem, E. Bosmans, A. Dekeersmaecker, G. Salebier, and B. Spitz, "Definition of a type of abnormal vaginal flora that is distinct from bacteria vagnosis: aerobic vaginitis B," *aerobic vaginitis B Jog,.*, vol. 109, pp. 34–43, 2002.

[4] Z. Ling, X. Liu, Y.W. X., L. Yuan, X. Tong, L. Li, and C. Xiang, "Associations between Vaginal Pathogenic Community and Bacterial Vaginosis in Chinese Reproductive –Age Women," pp. 1–8, 2013.

[5] C. Cartwright, B. Lernbke, K. Ramachandran, B. Body, M. Nye, C. Rivers, and J. Schwebkec, "Development and Validation of a Semi quantitative, Multi target PCR Assay for Diagnosis of Bacterial Vaginosis," *J. of Clinic. Microbiol*, vol. 50, pp. 2321–2329, 2014.

[6] D.N. Fredricks, T.L. Fiedler, K.K. Thomas, C. M. Mitchell, and J. Marrazzo, "Changes in Vaginal Bacterial Concentrations with Intravaginal Metronidazole Therapy for Bacterial Vaginosis as Assessed by Quantitative PCR," *J. Clin Microbiol*, vol. 47, pp. 721–726, 2009.

[7] P. A. Cadieux, J. Burton, E. Devillard, and G. Reid, "Lactobacillus By products Inhibit the growth and virulence of uropathogeni c Escherichia coli," *J physiol pharmacol*, vol. 12, pp. 12–16, 2009.

[8] D.G. Eerris, S.L. Francis, E.D. Dickman, K. Miler-Miles, I.T. Wailer, and N. MeClendon "Variability of vaginal pH determination by patients and clinicians. JAm Board Earn Med," *JAm Board Earn Med*, vol. 19, pp. 368–373, 2006.

[9] M. Fang, W. Tong, M. Schil, P. Blair, B. W. R., B. J. Hass, Q. Xie., S. L. M. Dial, G.I., and Shehan, "Structure activity relationship for a large diverse set of natural, synthetic and environment estrogens," *Chem. Res. Toxical*, vol. 14, no. 3, pp. 780–94, 2001.

[10] D.A., Eschenbach; S.S. Thwin, D.L. Patton, T.M. Hooton, A.E. Stapleton, K.K., Agnew., C., Winter; A., Meier and S. Stammow. "Influence of the normal menstrual cycle on vaginal tissue discharge and micro flora," *Clin. Infect. Dis*, vol. 30, pp. 901–907, 2000.

[11] J. Mashburn, "Etiology, diagnosis & management of vaginitis ," *J. of midwifery and women's health*, vol. 51, p. 423, 2000.

[12] N. Nikolailchouk, "The Female Genital Tract Micro biota Composition, Relation to Innate Immune Factors Effects of Contraceptives. Institute of biomedicine at Sahlgremska Academy," 2009.

[13] R.W. Hymen, M. Fukshima, L. Diamond, J. Kumm, L. C. Guidice, and R. Davis, "Microbes on the human vaginal epithelium," *P.A.NS*, vol. 102, no. 22, pp. 7952–7957, 2005.

[14] P. A. Mardh, "The vaginal ecosystem. ," *A.M J. obstet. Gynecol*, vol. 165, pp. 1163–1168, 1991.

[15] G.G. Donder, E. Bosmans, A. Dekeermaecker, A. Vereechem, B. vanbulch, and B. Spitz,

"Pathogeneses of abnormal vaginal bacteria flora," *AM. J. Ostet. Gynaecol*, vol. 182, pp. 872–878, 2000.

[16] L. Millier, D.L. Pahon, A. Meier, S.S. Thwin, T.M. Hooton, and T.M., Hooton; D.A., Escheh "Depomedroxy progesterone induced hypo estrogenism and change in vaginal flora and epithelium obstet," *Gynecol*, vol. 96, pp. 431–439, 2000.

[17] E. Mbizov, Msuyas, B. Stray-Pedersen, J. Sundby, M. Chirenje, and A. Hussain, "Determinate of reproductive treat infection among asymptomatic women in Harare, Zimbabwe," *Center Afr. J Med*,, vol. 47, no. 3, pp. 57–64, 2001.

[18] K. Backerman and D. J. Dudley, "Reproductive and immune system in perslow, Lenge medical books," in *Medical immunology*, A. T.G.; Sites, D.P.; Terr and I. J.B., Eds. New York: McGraw Hill publishing Division, 2001, pp. 19–43.

[19] R.M. Brotman, M.. Klebanoff, T. Nansel, and et al., "Alongtudinal study of vaginal douching and Bacterial vaginosis a marginal structural Modeling analysis," *A m J Epidemiol*, vol. 168, pp. 188–96, 2008, 188 – 96.

[20] P.G. Larsson, B. Carisson, L. Fhraeus, T. Jakobsson, and U. Forsum, "Diagnosis of bacterial vaginosis: need for validation of microscopic image area used for scoring bacterial morph types," *Sex Transm Infect*, vol. 80, pp. 63–67, 2004.

[21] S. Smart, A. Singal, and A. Mindel, "Social and sexual Risk factors for Bacterial vaginosis," *Sex Transm infect*, vol. 80, pp. 58–62, 2004.

[22] M. Pirotta, "Effect of lactobacillus in prevent post-antibiotic vulvovaginal candidasis. Arandomised controlled trial," *BMJ*, vol. 329, no. 7465, pp. 527–548, 2004.

[23] V. Lenjekar, N. Marathe, V. Ramana, Y. Shonche, and D. Ranade, "Megasphaera sp . nov. , an obligate anaerobic bacteria isolated from human faces" pp. 2250–2256, 2014.

[24] E. Shipitsyna, A. Roos, R. Datcu, A. Hallen, H. Fredlund, J. S. Jensen, L. Engstrand, and M. Unemo, "Composition of the vaginal micro biota in women of reproductive age – sensitive and specific molecular diagnosis of bacterial vaginosis is possible?" *PLOS*, vol. 8, 2013.

[25] R.H. Verhelst, G. Verstraelen, G. Claeys, J. Verschraegen, L. Delanghe, C. V. Simaey, M. D. Ganck, Temmerman, and M. Vaneechoutte, "Cloning of 16 S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong between Atopobium vaginae , Gardnerella vaginallis and bacterial vaginosis," *BMC Microbiol*, vol. 4, p. 16, 2004.

[26] G.G. Donder, E. Bosmans, A. Dekeermaecker, A. Vereechem, B. vanbulch, and B. Spitz, "Pathogeneses of abnormal vaginal bacteria flora," *AM. J. Ostet. Gynaecol*, vol. 182, pp. 872–878, 2000.

[27] A. Haikara, "The genus pectinatus and Megasphaera In: The prokaryotes," B. A, H. Triiper, M. Dworkin, W. Marder, and K.H. Sebleifer, Eds., vol. 2. New York: Springer–verlag, pp. 1993–2004m 1991.

[28] A. Vásquez, T. Jakobsson, S. Ahrné, U. Forsum, and G. Molin, "Vaginal Lactobacillus Flora of Healthy Swedish Women," *J Clin Microbiol*, vol. 40, pp. 2746–2749, 2002.

[29] X. Zhou, M.A. Hansmann, C. Davis, H. . Suzuki, C. J. Brown, U. Schutte, J. D. Pierson, and L. J. Forney, "The vaginal Bactenial communities of Japanese women resemble those of women in other racial group. FEMS immunol Med Microbiol," *FEMS immunol Med Microbiol*, vol. 58, pp. 169–181, 2010.

[30] C.S. Bradshaw, A.N. Morton, J. Hocking, S.M. Garland, M.B. Morris, L.M. Moss, L.B. Horvath, I. Kuzevska, and C. Fairley, "Hight Recurrence Rates of Bacterial vagnosis over the course of 12 Months after oral Metronidazole therapy and factors Associated with" 2006.

[31] M.A. Krohn, S.L. Hillier, and D.A. Eschenbach, "Comparison of methods for diagnosing bacterial vaginosis among pregnant," *J Clin Microbiol*, vol. 27, pp. 1266–1271, 1989.

[32] D. Fredricks, T. . Fiedler, and J. Marrazzo, "Molecular identification of bacteria associated with bac- terial vaginosis," *N . Engl– J. Med* ., vol. 353, pp. 1899–1911, 2005.

[33] M.Z. Minchliffe, D. Martin, and M. Ferris, "Prevalence and abundance of uncultivated

Megasphaera like Bacteria," pp. 656–1659, 2008.

[34] C. Muzny, I. Sunesara, M. Griswold, R. Kumar, E. Lelkowitz, L. Mena, J. Schwebke, D. Martin, and E. Swiatlo, "Association between BVAB 1 and high Nugent scores among women with bacterial vaginosis .Diag, Microbiol," *Diag, Microbiol. and Infect. Dis*, vol. 10, pp. 1–3, 2014.

[35] E. Shipitsyna, A. Roos, R. Datcu, A. Hallen, H. Fredlund, J.S. Jensen, L. Engstrand, and M. Unemo, "Composition of the vaginal micro biota in women of reproductive age – sensitive and specific molecular diagnosis of bacterial vaginosis is possible ?" *PLOS*, vol. 8, 2013.

[36] Z. Ling, X. Liu, Y. Lou, X. Wu, L. Yuan, X. Tong, L. Li, and C. Xiang, "Associations between Vaginal Pathogenic Community and Bacterial Vaginosis in Chinese Reproductive–Age Women," pp. 1–8, 2013.

[37] M.T. Madigan, J. Martinko, and J. Parker, "Brook Biology of ' Microorganisms." New Jersey: Pearson Education, Upper Saddle River, 2003, pearson Education, Upper Saddle River,.

[38] A. Mlorrot, E. Grapádo, A. Pe'rez, S. Barbosa, S. Silva-Barbosa, N. Milicevic, and D. F. de Oliveira, *Chagasic Thymic Atrophy Does Not Affect Negative Selection but Results*, 2011, vol. 5.

[39] W.E. Trick, S.K. Fridkin, J.R. Edwardsm, R.A. Hajjeh, and R.P. Gaynes, "Secularltrend of hospital– acquired candidemia among intensive care unit patients in the United State during 1989," *Clin .. Infect . Dis.*, vol. 35, pp. 627–630, 2002.

[40] M. Zozaya-Hinchliffe, R. Lillis, D. Martinand, and M. Ferris, "Quantitative PCR assessments of Bacte- rial Species in Women with and without Bacterial Vaginosis," *J Clin Microbiol*, vol. 48, pp. 1812–1819, 2010.

[41] S. Graham, C. Howes, R. Dunsmuir, and J. Sandoe, "Vertebral ostemyelitis and discitis due to Gard- nerella vaginalis," *J Med Microbiol*, vol. 58, pp. 14 382–1384, 2009.

[42] T. Vos, A. D. Flaxaman, M. Naghavi, R. Lozano, C. Michaud, and M. Ezzati, "Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010 :a systematic analysis for the Global Burden of Disease Study 2010n" pp. 2163 –2196, 2012.

[43] W. Zeger and K. Holt, "Gynaecological infections. Emerg Medicine," *North Am*, vol. 21, no. 3, pp. 631–48, 2003.

[44] R.W. Hymen, M. Fukshima, L. Diamond, J. Kumm, L.C. Guidice, and R. Davis, "Microbes on the human vaginal epithelium," *P.A.NS*, vol. 102, no. 22, pp. 7952–7957, 2005.

[45] J.P. Menard, F. Fenollar, M. Henry, F. Bretelle, and D. Raoult, "Molecular quantification of Gardnerella vaginalis and Atopobium vaginae loads to predict bacterial vaginosis . Clin Infect Dis," *Clin Infect Dis*, vol. 47, pp. 33–43, 2008.

[46] D.N. Fredricks, T.L. Fedler, K. K. Thomas, B. Oakely, and J.M. Marrazzo, "Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis," *N. Engl– J. Med.*, vol. 45, pp. 3270–3276, 2007.

[47] R. Datcu, D. Gesink, J. Mulvad, R.M. Andersen, E. Rink, A. Koch, P. Ahrens, and J. Jensen, "Vaginal microbiome in women from Greenland assessed by microscopy and quantitative PCR," *BMC Infectious Diseases*, vol. 13, pp. 1–4, 2013.

[48] J.M. Marrazzo, T.L. Fiedler, S. Srinivasan, K.K. Thomas, C. Liu, D. Ko, H. Xie, M. Saracino, and D.N. Fredricks, "Extra vaginial reservoirs of vaginal bacteria as risk factors for incident bacterial vaginosis. 205:1580-1588," *J. Infect Dis*, vol. 205, pp. 1580–1588, 2012.

[49] T. Dumonceaux and et al., "Multilex detection of bacteria associated with normal microbiota and with bacterial vaginosis in vaginal swabs by use of oligonucleotide-coupled fluorescent microspheres." *J. of Clinic. Microbiol*, vol. 47, pp. 4967–4977, 2009.

الخلاصة

تم جمع (100) عينة من النساء المصابات بداء المشعرة المهبلية من مستشفيات محافظة بابل و العيادات الخاصة كل العينات تم جمعها من النساء الحوامل كان معدل عمر المريضات يتراوح ما بين (40-17) سنة العينة تم جمعها بواسطة اداة قطنية معقمة و هذه العينات تم خزنها في وضع التجميد لحين استعمالها و تم استخلاص المادة النووية الوراثية من هذه العينات المادة الوراثية القياسية للجينيوم تم تحضيرها للمادة الرايبوزية منقوصة الاوكسجين بعد استعمال المطياف الضوئي لتشخيص المسبب لهذا الداء بواسطة تقنيات تفاعلات البلمرة المتكررة اظهرت النتائج ان الفطريات كانت هي 22.92 % و اظهرت الدراسة دور عوامل الاصابة بالاجهاض و **Trichomonas** بينما **candida albicans** متمثلة بال **%**السائدة بنسبة 79.17 كانت لها عامل خطورة قوي مع الاجهاض .**Megasphaera.sp**اظهرت النتائج ان

الكلمات الدالة: المشعرة المهبلية , تفاعل التسلسل المتبلمر النوعي , الاجهاض