

Molecular investigation of vaginal bacteria in preterm birth women

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ARTICLE INFO

Received: July 15, 2024

Accepted: August 20, 2024

Volume: 4

Issue: 2

KEYWORDS

16S rRNA gene, Bacterial vaginosis, PCR, Pregnancy, Iraq

ABSTRACT

The current study was aimed to molecular investigation five species of bacterial vaginosis in preterm birth women including *Gardnerella vaginalis*, *Megasphaera vaginalis*, *Mobiluncus curtisii*, *Prevotella*, and *Facklamia*. A total of 90 preterm birth women of different ages were selected from different governmental and private hospitals in Wasit province (Iraq). Vaginal swabs were collected from each woman under aseptic conditions into plastic tubes to be examined molecularly using the conventional polymerase chain reaction (PCR). Targeting the *16S rRNA* gene, 6 different bacterial species were identified molecularly using the PCR assay in 58.89% vaginal swabs of preterm birth women. Among the infected women, significant increase ($p \leq 0.0175$) in bacterial species was seen in mixed infections (64.15%) when compared to single infections (35.85%). This study reported that the infection rate of bacterial species in single infections was increased significantly ($p \leq 0.0323$) for *Gardnerella vaginalis* (57.89%) infection and decreased significantly for *Prevotella* spp. (5.26%) and *Facklamia* spp. (5.26%) infections when compared to others; *Megasphaera vaginalis* (21.05%), and *Mobiluncus curtisii* (10.53%). For mixed infections, significant increase ($p \leq 0.0235$) in values of mixed infection was observed in *G. vaginalis* and *M. vaginalis* (38.24%) while decrease was recorded in *M. curtisii* and *Prevotella* spp. (2.94%); *Prevotella* spp. and *Facklamia* spp. (5.88%); *G. vaginalis*, *M. vaginalis* and *M. curtisii* (2.94%); as well as *Prevotella* spp., *Facklamia* spp. and *M. curtisii* (2.94%). In conclusion, many bacterial species might be implicated in incidence of vaginosis in preterm birth women in particular *Gardnerella vaginalis* that detected significantly in both single and mixed infections. This suggests that additional studies are necessary to identify the possible role for each bacterial infection in various pregnancy disorders.

1. Introduction

Pregnancy is a complex event that is fraught with risks and causing a stress to mother. Preterm birth is a major problem hampers practice of pregnancy and causes several mortalities and morbidities, especially in ectopic pregnancies (Davis and Narayan, 2020; Orsi et al., 2023). It usually starts with the last menstrual period and continues until delivery at about 40 weeks. Possible symptoms include the lack of menstruation (amenorrhea), incontinence, behavioral, cervical, and vaginal change, as well as restlessness (Shaffron and Watkins, 2022). In addition, some complications might be occurred such as vaginal bleeding, persistent vomiting, chills or fever, vaginal discharge, abdominal or chest pain, high blood pressure, increased or decreased fetal movements (Doumouchtsis et al., 2016). However, the prevalence of preterm birth varies worldwide as estimated at 11.1% of all births worldwide, but in most developed European countries it reaches 5%, and in some African countries it reaches 18% (Ikeoha et al., 2022). In a recent study, it's estimated that premature births cause the death of nearly one million children under the age of five (Lawn et al., 2023). Although the causes are not clear, the main causes of the disease are the development of diseases in the uterus and the inflammatory response of the fetus which including uterine tissue damages, and fetal ruptures, and development of uterine structural adjustments (Crump, 2020; Menon et al., 2020). Furthermore, preterm infants have a poor immune response, including reduced levels of IgG with elevation of inflammatory markers like interleukins (ILs) and tumor-necrosis factor (TNF) in various cases might be related to the pathogenesis of preterm birth as have been shown to that these markers may act as a predictive markers of preterm birth in asymptomatic and symptomatic women (Shah and Baxi, 2016; Helmo et al., 2018; Denney et al., 2021).

Bacterial vaginosis is a common vaginal infection that happens when some normal vaginal flora overgrow causing unusual discharge, discomfort, and pain with replacement of normal hydrogen peroxide (pH) (Donders et al., 2014; Tomas et al., 2020). Several bacteria are associated with bacterial vaginosis such as *Gardnerella* spp., *Megasphaera* spp., *Mobiluncus* spp., *Prevotella* spp., *Sneathia* spp., and mixed vaginal anaerobic species with many species facilitating growth and relapsing of beneficial *Lactobacilli* that help maintain a healthy vaginal environment (Chee et al., 2020; Deng et al., 2022). There are also high concordances of bacterial vaginosis-associated bacterial species among sexual partners (Forcey et al., 2015). For diagnosing of bacterial species, different laboratory tests can be conducted such as traditional culture and microscopic examination of Giemsa's stained-slides and advanced molecular techniques that based on nucleic acid amplification such as polymerase chain reaction (PCR) assay (Leal Jr et al., 2021).

The current study was aimed to molecular investigation five species of bacterial vaginosis in preterm birth women of Wasit province (Iraq) including *Gardnerella vaginalis*, *Megasphaera vaginalis*, *Mobiluncus curtisii*, *Prevotella*, and *Facklamia*.

2. Methodology

Ethical Approval

This study licensed by the Scientific Committee of the College of Density (University of Wasit).

Samples

A total of 90 preterm birth women of different ages were selected to the present study that conducted during March (2023) to February (2024) at different governmental and private hospitals in Wasit province (Iraq). Initially, each woman was subjected to direct obtaining of vaginal swabs under aseptic conditions into plastic tubes that kept cooled and transported to the lab using an ice-box for molecular examination.

Molecular examination

Genomic DNAs were extracted from the swab samples as described in manufacturers' instructions of the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). After evaluation of concentration and purity by the Nanodrop system (Thermo Scientific, UK), GoTaq® Green Mastermix Kit (Promega, Korea) was served to preparing the Mastermix tubes at a total volume of 25µl. Targeting 16S rRNA gene of each bacterial species, the primers were designed based on the GenBank isolates (Table 1). Following specified conditions for each primer in the Thermal Cycler system (BioRad, USA), PCR reaction was conducted. Then, electrophoresis of the PCR products in agarose-gel (1.5%) stained with Ethidium bromide was performed at 80 Am and 100 Volt for 90 min. and the product sizes of analysed samples were visualized under the UV transilluminator.

Table 1. List of primers designed for detection vaginal bacterial species by targeting 16S rRNA gene

Species		Primer sequence	Product size	GenBank ID
<i>Gardnerella vaginalis</i>	F	GCTCTTGGAAACGGGTGGTA	523 bp	OP629599.1
	R	CCGTTACACCGGGAATTCCA		
<i>Megasphaera vaginalis</i>	F	GGAAGTGGACACGGTCCAG	591 bp	NR_179646.1
	R	TCAACCTTGCGGTCTACTC		
<i>Mobiluncus curtisii</i>	F	ATACCCTGGTAGTCCACGCT	555 bp	NR_074732.1
	R	AGCGACTCCAACCTTCATGGG		
<i>Prevotella</i> spp.	F	GAGAGCCTGAACCAGCCAAG	402 bp	MN665355.1
	R	CCTGTTTCGATACCCGCACTT		
<i>Facklamia</i> spp.	F	TCAACCGTGGAGGGTCATTG	554 bp	LT971014.1
	R	GGTTTGTCACCGGCAGTCTA		

Statistical analysis

The t-test in the GraphPad Prism Software was applied to detect significant differences between the obtained values at $P < 0.05$ (Gharban et al., 2023).

3. Results

Targeting the 16S rRNA gene, five different bacterial species were identified molecularly using the PCR assay in 53 (58.89%) vaginal swabs of preterm birth women (Figure 1). Among the infected women, significant increase ($p \leq 0.0175$) in bacterial species was seen in mixed infections [34 (64.15%)] when compared to single infections [19 (35.85%)] (Figure 2).

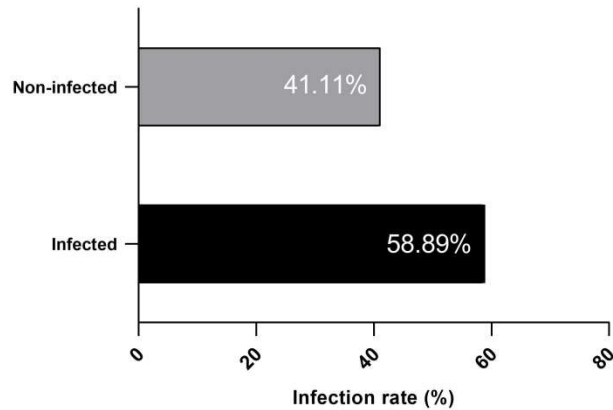


Figure 1. Total infection rate of 6 bacterial species in totally 90 preterm birth tested women.

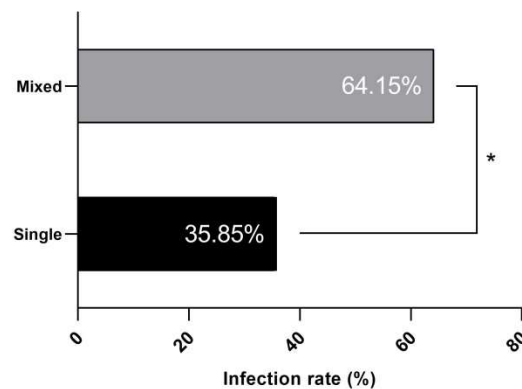


Figure 2. Total infection rate of 6 bacterial species in totally 90 preterm birth tested women.

This study reported that the infection rate of bacterial species in single infections was increased significantly ($p \leq 0.0323$) for *Gardnerella vaginalis* (57.89%) infection and decreased significantly for *Prevotella* spp. (5.26%) and *Facklamia* spp. (5.26%) infections when compared to others; *Megasphaera vaginalis* (21.05%), and *Mobiluncus curtisii* (10.53%), (Table 2).

Table 2. Infection rate of bacterial species among totally 19 single infected preterm birth women

Species	No.	%
<i>Gardnerella vaginalis</i>	11	57.89 *
<i>Megasphaera vaginalis</i>	4	21.05
<i>Mobiluncus curtisii</i>	2	10.53
<i>Prevotella</i> spp.	1	5.26
<i>Facklamia</i> spp.	1	5.26
Discrepancy		16.67
SD		13.89
SEM		5.672
<i>t</i>		2.938
p-value		0.0323
Significance		Yes (*)

95% Confidence interval	14.58 (2.086 to 31.25)
R squared	0.6333

For mixed infections, significant variation was recorded in infection rates of different bacterial species. Significantly, the higher value of mixed infection was detected in infected women with only 2 bacterial species [32 (94.12%)] more than the infected women with 3 bacterial species (5.88%), (Figure 3).

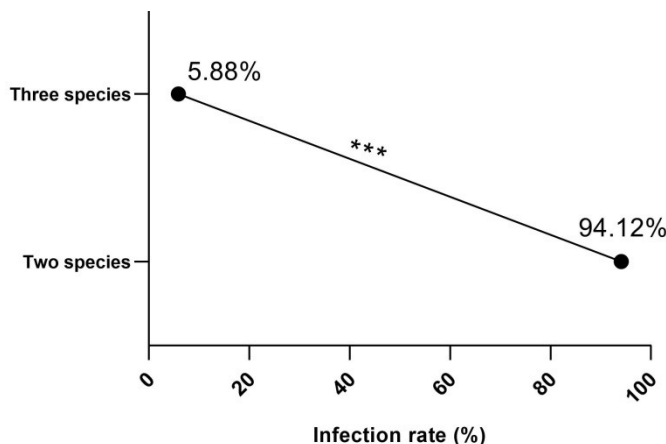


Figure 3. Prevalence rate of mixed infections among preterm infected women (total no: 34)

Subsequently, significant increase ($p \leq 0.0235$) in values of mixed infection was observed in *G. vaginalis* and *M. vaginalis* (38.24%) while decrease was recorded in *M. curtisii* and *Prevotella* spp. (2.94%); *Prevotella* spp. and *Facklamia* spp. (5.88%); *G. vaginalis*, *M. vaginalis* and *M. curtisii* (2.94%); as well as *Prevotella* spp., *Facklamia* spp. and *M. curtisii* (2.94%), (Table 3).

Table 3. Infection rate of bacterial species among totally 34 mixed infected preterm birth women

Species	No.	%
<i>G. vaginalis</i> + <i>M. vaginalis</i>	13	38.24 *
<i>G. vaginalis</i> + <i>Prevotella</i> spp.	5	14.71
<i>G. vaginalis</i> + <i>Mobiluncus curtisii</i>	7	20.59
<i>M. vaginalis</i> + <i>Mobiluncus curtisii</i>	4	11.76
<i>M. curtisii</i> + <i>Prevotella</i> spp.	1	2.94
<i>M. curtisii</i> + <i>Facklamia</i> spp.	2	5.88
<i>G. vaginalis</i> + <i>M. vaginalis</i> + <i>M. curtisii</i>	1	2.94
<i>Prevotella</i> spp. + <i>Facklamia</i> spp. + <i>M. curtisii</i>	1	2.94
Discrepancy		12.5
SD		12.26
SEM		4.33
<i>t</i>		2.885
p-value		0.0235
Significance		Yes (*)
95% Confidence interval		10.25 (2.254 to 22.75)
R squared		0.5431

3. Discussion

Bacterial vaginosis is a condition characterized by an imbalance of bacteria in the vagina, leading to changes in the natural bacteria levels in addition to various disturbances even abortion (Donders et al., 2000; Carlsson et al., 2018). In this study, 58.89% of study preterm birth women were found positively infected by different bacterial species; in which, significant higher infections were seen with *Gardnerella vaginalis* in both single and mixed infections. The elevated prevalence rate of bacterial vaginosis might be attributed to high sensitivity and specificity of PCR assay in detection of different microorganisms. PCR has several advantages, including its ability to produce rapid results, allowing for quicker diagnosis and optimal treatment decisions. It supports antimicrobial stewardship by avoiding unnecessary antibiotics and can test for multi-drug resistance, effectively

identifying pathogens like MRSA. Additionally, PCR is a simple and user-friendly technique (Teymouri et al., 2021; Gharban, 2024).

In many previously and recently conducted studies, diagnostic criteria were based on culture. Bacterial culture methods have several disadvantages, including a lengthy and tedious culturing process, a high susceptibility to contamination, and the fact that they often require more training compared to molecular techniques. Additionally, certain bacterial cultures are not suitable for long-term storage as some organisms may lose viability or undergo changes in their properties over time (Marrazzo et al., 2010; Tomas et al., 2020). In a study in Columbia, only 6.66% of the vaginal swabs were isolated on blood agar; whereas, targeting the *16S rRNA* gene, 62.66% were successfully identified (Raphael et al., 2011). In comparison to several studies, it's estimated that the incidence *Gardnerella vaginalis* is ranged 20 to 60% (Shipitsyna et al., 2019), while in other countries, it shows that the infection rate in women varies from 5-70% (Lanza et al., 2023). In the United States, Australia, New Zealand and Western Europe have the lowest number of incidents with considering that *Gardnerella* species are the main pathogens of bacterial vaginosis (Frimodt-Møller et al., 2023). In another study by Ng et al. (2023), *Gardnerella vaginalis* was isolated in 66.7% of positive bacterial vaginosis group with a rate of preterm births ≤ 34 weeks increased significantly (22.7% vs. 6.2%). However, the speed with which this bacterium can be isolated in patients with bacterial vaginosis depends on the diagnostic technique, method of samples collection, and diagnostic criteria (Redelinguys et al., 2020).

In a study conducted by Srinivasan et al. (2023), the results showed that *Megasphaera vaginalis* was useful for detection of different pathogens of bacterial vaginosis and has been included as a target in commercial bacterial vaginosis is nucleic acid amplification tests. In addition, the probability of spontaneous delivery is higher in pregnant women with a history of childbirth and a higher number of *Megasphaera vaginalis* in 2nd trimester. An association between *Megasphaera vaginalis* and premature birth was also confirmed in a case-control study of African American women (Florova et al., 2021). Ravel et al. (2020) showed that women with pelvic inflammatory disease were more likely to test positive for *Megasphaera vaginalis* and other anaerobic bacteria. McClelland et al. (2018) *Megasphaera vaginalis* species have recorded an association to HIV infection in South African women. Our findings seem to contradict the findings of Kumar et al. (2021) as the vaginal swabs collected from the 3rd trimester of 38 Indian pregnant women with spontaneous labor delivery have largely *Megasphaera vaginalis* in vaginal mucosa

4. Conclusion

Many bacterial species might be implicated in incidence of vaginosis in preterm birth women in particular *Gardnerella vaginalis* that detected significantly in both single and mixed infections. Also, the demographic characteristics of the patients such as age, hygiene habits, number of pregnancies, and chronic diseases (e.g. diabetes, urinary tract infections, and renal failure) could play a role in increasing of infection and reproductive disorders. This suggests that additional studies are necessary to identify the possible role for each bacterial infection in various pregnancy disorders.

References

- [1]. Carlsson, I., Breeding, K., and Larsson, P. G. (2018). Complications related to induced abortion: a combined retrospective and longitudinal follow-up study. *BMC women's health*, 18, 1-9.
- [2]. Chee, W. J. Y., Chew, S. Y., and Than, L. T. L. (2020). Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. *Microbial cell factories*, 19(1), 203.
- [3]. Crump, C. (2020). Preterm birth and mortality in adulthood: a systematic review. *Journal of Perinatology*, 40(6), 833-843.
- [4]. Davis, E. P., and Narayan, A. J. (2020). Pregnancy as a period of risk, adaptation, and resilience for mothers and infants. *Development and psychopathology*, 32(5), 1625-1639.
- [5]. Deng, T., Shang, A., Zheng, Y., Zhang, L., Sun, H., and Wang, W. (2022). Log (Lactobacillus crispatus/Gardnerella vaginalis): a new indicator of diagnosing bacterial vaginosis. *Bioengineered*, 13(2), 2981-2991.
- [6]. Denney, J. M., Nelson, E., Wadhwa, P., Waters, T., Mathew, L., Goldenberg, R. L., and Culhane, J. F. (2021). Cytokine profiling: variation in immune modulation with preterm birth vs. uncomplicated term birth identifies pivotal signals in pathogenesis of preterm birth. *Journal of perinatal medicine*, 49(3), 299-309.
- [7]. Donders, G. G., Van Bulck, B., Caudron, J., Londers, L., Vereecken, A., and Spitz, B. (2000). Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. *American journal of obstetrics and gynecology*, 183(2), 431-437.

- [8]. Donders, G. G., Zodzika, J., and Rezeberga, D. (2014). Treatment of bacterial vaginosis: what we have and what we miss. *Expert opinion on pharmacotherapy*, 15(5), 645-657.
- [9]. Doumouchtsis, S. K., Hordern, C., Mukhopadhyay, S., Papageorghiou, A., Tamizian, O., and Watt-Coote, I. (2016). Medical emergencies in pregnancy. *Emergencies in Obstetrics and Gynaecology*, 21.
- [10]. Florova, V., Romero, R., Tarca, A. L., Galaz, J., Motomura, K., Ahmad, M. M., and Gomez-Lopez, N. (2021). Vaginal host immune-microbiome interactions in a cohort of primarily African-American women who ultimately underwent spontaneous preterm birth or delivered at term. *Cytokine*, 137, 155316.
- [11]. Forcey, D. S., Vodstrcil, L. A., Hocking, J. S., Fairley, C. K., Law, M., McNair, R. P., and Bradshaw, C. S. (2015). Factors associated with bacterial vaginosis among women who have sex with women: a systematic review. *PloS one*, 10(12), e0141905.
- [12]. Frimodt-Møller, N., Nielsen, K. L., and Hertz, F. B. (2023). The Urinary and Vaginal Microbiomes. In *Textbook of Female Urology and Urogynecology* (pp. 598-608). CRC Press.
- [13]. Gharban, H.A., Al-Shaeli, S.J., and Hussien, T.J. (2023). Molecular genotyping, histopathological and immunohistochemical studies of bovine papillomatosis. *Open Veterinary Journal*, 13(1), 26-41.
- [14]. Gharban, H.A. (2024) First genotyping confirmation of *Pichia kudriavzevii* in subclinically mastitic cows in Iraq. *Rev. Ciênc. Agrovet.*, 23 (3): 529-536
- [15]. Helmo, F. R., Alves, E. A. R., Moreira, R. A. D. A., Severino, V. O., Rocha, L. P., Monteiro, M. L. G. D. R., and Corrêa, R. R. M. (2018). Intrauterine infection, immune system and premature birth. *The journal of maternal-fetal and neonatal medicine*, 31(9), 1227-1233.
- [16]. Ikeoha, C. C., Anikwe, C. C., Umeononihu, O. S., Okoro-chukwu, B. C., Mamah, J. E., Eleje, G. U., and Ugwoke, I. S. (2022). Fetal Fibronectin as a Predictor of Preterm Delivery: A Nigerian Cohort Study. *BioMed Research International*, 2022(1), 2442338.
- [17]. Kumar, S., Kumari, N., Talukdar, D., Kothidar, A., Sarkar, M., Mehta, O., and GARBH-Ini Study Group. (2021). The vaginal microbial signatures of preterm birth delivery in Indian women. *Frontiers in cellular and infection microbiology*, 11, 622474.
- [18]. Lanza, M., Scuderi, S. A., Capra, A. P., Casili, G., Filippone, A., Campolo, M., and Paterniti, I. (2023). Effect of a combination of pea protein, grape seed extract and lactic acid in an in vivo model of bacterial vaginosis. *Scientific Reports*, 13(1), 2849.
- [19]. Lawn, J. E., Bhutta, Z. A., Ezeaka, C., and Saugstad, O. (2023). Ending preventable neonatal deaths: multicountry evidence to inform accelerated progress to the sustainable development goal by 2030. *Neonatology*, 120(4), 491-499.
- [20]. Leal Jr, S. M., Rodino, K. G., Fowler, W. C., and Gilligan, P. H. (2021). Practical guidance for clinical microbiology laboratories: diagnosis of ocular infections. *Clinical microbiology reviews*, 34(3), 10-1128.
- [21]. Marrazzo, J. M., Martin, D. H., Watts, D. H., Schulte, J., Sobel, J. D., Hillier, S. L., and Fredricks, D. N. (2010). Bacterial vaginosis: identifying research gaps proceedings of a workshop sponsored by DHHS/NIH/NIAID. *Sexually transmitted diseases*, 37(12), 732-744.
- [22]. McClelland, R. S., Lingappa, J. R., Srinivasan, S., Kinuthia, J., John-Stewart, G. C., Jaoko, W., and Fredricks, D. N. (2018). Key vaginal bacteria associated with increased risk of HIV acquisition in African women: A nested case-control study. *The Lancet. Infectious diseases*, 18(5), 554.
- [23]. Menon, R., Behnia, F., Poletini, J., and Richardson, L. S. (2020, August). Novel pathways of inflammation in human fetal membranes associated with preterm birth and preterm pre-labor rupture of the membranes. In *Seminars in immunopathology*. Berlin/Heidelberg: Springer Berlin Heidelberg, 42, (4), 431-450.
- [24]. Ng, B. K., Chuah, J. N., Cheah, F. C., Mohamed Ismail, N. A., Tan, G. C., Wong, K. K., and Lim, P. S. (2023). Maternal and fetal outcomes of pregnant women with bacterial vaginosis. *Frontiers in Surgery*, 10, 1084867.
- [25]. Orsi, M., Jannah, F. M., Sesay, A., Bah, A. K., and Tiru, N. A. (2023). A Clinical and Ethical Dilemma: Expectant Management for Ectopic Pregnancy with a Vital Fetus in a Low-Resource Setting. *Journal of Clinical Medicine*, 12(17), 5642.
- [26]. Raphael, E., Madhivanan, P., Reingold, A., Klausner, J., Riley, L., and Trammell, S. (2011). P3-S7. 19 Diversity of the vaginal flora determined by molecular methods in healthy women and women with bacterial vaginosis in the Bay Area, California, USA. *Sexually Transmitted Infections*, 87(Suppl 1), A305-A306.
- [27]. Ravel, J., Moreno, I., and Simón, C. (2021). Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. *American journal of obstetrics and gynecology*, 224(3), 251-257.
- [28]. Redelinghuys, M. J., Geldenhuys, J., Jung, H., and Kock, M. M. (2020). Bacterial vaginosis: current diagnostic avenues and future opportunities. *Frontiers in cellular and infection microbiology*, 10, 354.
- [29]. Shaffron, M., and Watkins, E. (2022). Behavioral Health in Obstetrics and Gynecology. *Physician Assistant Clinics*, 7(3), 465-475.
- [30]. Shah, J., and Baxi, B. (2016). Identification of biomarkers for prediction of preterm delivery. *Journal of Medical society*, 30(1), 3-14.

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- [31]. Shipitsyna, E., Krysanova, A., Khayrullina, G., Shalepo, K., Savicheva, A., Guschin, A., and Unemo, M. (2019). Quantitation of all four Gardnerella vaginalis clades detects abnormal vaginal microbiota characteristic of bacterial vaginosis more accurately than putative G. vaginalis sialidase A gene count. *Molecular diagnosis and therapy*, 23, 139-147.
- [32]. Srinivasan, S., Austin, M. N., Fiedler, T. L., Strenk, S. M., Agnew, K. J., Gowda, G. N., and Hillier, S. L. (2023). Amygdalobacter indicium gen. nov., sp. nov., and Amygdalobacter nucleatus sp. nov., gen. nov.: novel bacteria from the family Oscillospiraceae isolated from the female genital tract. *International Journal of Systematic and Evolutionary Microbiology*, 73(10), 006017.
- [33]. Teymouri, M., Mollazadeh, S., Mortazavi, H., Ghale-Noie, Z. N., Keyvani, V., Aghababaei, F., and Mirzaei, H. (2021). Recent advances and challenges of RT-PCR tests for the diagnosis of COVID-19. *Pathology-Research and Practice*, 221, 153443.
- [34]. Tomas, M., Palmeira-de-Oliveira, A., Simoes, S., Martinez-de-Oliveira, J., and Palmeira-de-Oliveira, R. (2020). Bacterial vaginosis: Standard treatments and alternative strategies. *International journal of pharmaceutics*, 587, 119659.