



### Original Article

# Aerobic Bacterial Vaginosis and *Lactobacillus* Species Associated with Cytomegalovirus in Abortion

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## Abstract

Abortion is a common complication in the life cycle of pregnancy. Based on the American College of Obstetricians and Gynecologists, spontaneous abortion is defined as the expulsion of an embryo or the extraction of a fetus at the age of 20-22 weeks of pregnancy. This study aimed to investigate the relationship between socioeconomic factors and bacterial vaginosis (BV) in women having an abortion. As a secondary aim, it attempted to detect common bacteria that cause vaginosis associated with miscarriage and are related to Cytomegalovirus (CMV) and *Lactobacillus* species (spp.). A total of 113 high vaginal swabs were taken from women having an abortion. Some variables that have been investigated in this study include age, education, and infection. After the vaginal discharge collection, the smear was prepared. Afterward, one or two drops of normal saline solution were put on the prepared smear with a cover slip, and then, they were examined under a microscope. Gram stain kits (Hi-media, India), were used to differentiate between the shapes of bacterial isolates. The wet mount technique was then utilized for the detection of *Trichomonas vaginalis* and aerobic BV. All the samples were used for smear gram staining and were cultured on blood agar, chocolate agar, as well as MacConkey agar. Biochemical examinations performed on suspicious cultures included the Urease test, Oxidase test, Coagulase test, and Catalase test. In the present study, the participants' age ranged from 14 to 45 years. Women aged 24-34 years had a high rate of miscarriage, determined at 48 (42.5%), which was considered a high incidence rate. The results showed that 28.6% of the studied population experienced abortion once and 71.4% of them experienced it twice due to aerobic BV. The recorded data also revealed that 50% of the studied population, who were infected with CMV or *Trichomonas vaginalis*, experienced abortion once and the other 50% experienced it twice. From 102 samples infected with *Lactobacillus* spp., 45.17% experienced abortion once and 42.2% experienced it twice.

**Keywords:** Bacterial vaginosis, Recurrent miscarriage, Type of contraceptive, Vaginal discharge

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## 1. Introduction

The link between microbiota and human health has attracted scientists' attention, and a growing number of studies, as well as research projects, have been conducted on this issue. Since a decade ago, the Human Microbiome Project and the European MetaHIT consortium have been initiated aiming to characterize the structure and composition of microbiota from various body sites. First, the composition and function of the gastrointestinal (GI) microbiota attracted the

attention to find the relationship between health status and microbiota. However, attention has also been paid to microbiota from other sites of the living body, such as the genital tract. The genital tract is colonized by microorganisms known as the vaginal microbiota (VMB).

Miscarriage is one of the most common difficulties women face during the first stage of pregnancy, which is not only linked to morbidity and death but also affects women socially, as well as psychologically (1).

Some factors are considered responsible for the higher percentages of miscarriage occurrence, which include mothers' ethnic origin, psychological state, stress level, the consumption of non-steroidal anti-inflammatory medicine, smoking, and alcohol (1). Bacterial vaginosis (BV) is a common vaginal infection in women of childbearing age, which is a condition of an imbalance in the vaginal flora. At the age of prepuberty, the vaginal flora is composed of several bacteria, such as *Diphtheroid*, *Staphylococcus epidermidis*, *Streptococci*, and *Escherichia* (2). Although BV is not a sexually transmitted infection, it has been associated with an increased risk of sexually transmitted diseases. Furthermore, it has been considered a risk factor for negative pregnancy outcomes, including premature labor, recurrent miscarriages, post-abortion sepsis, early miscarriage, and stillbirth (3).

The VMB is an important factor in protecting the host from various bacterial, fungal, and viral pathogens. In addition, the mother's VMB plays an essential role in the initial colonization of newborns, and thus, the development of healthy GI and skin microbiota. Therefore, the present investigated the BV and *Lactobacillus* species (spp.) associated with Cytomegalovirus (CMV) in abortion.

## 2. Materials and Methods

### 2.1. Sample Collection

A total of 113 high vaginal swabs were obtained from females (aged 15-45) being attended to the Obstetrics-Gynecology Patients Department in AL Sadder Hospital (Misan, Iraq) and the College of Sciences in Misan University (Amarah, Iraq) from November 2019 to May 2020. High vaginal swabs were taken from females based on the symptoms of vaginosis, vaginal discharge, itching, bad odor, and other clinical manifestations. For sampling, a sterile metal speculum was inserted into the vagina without any lubricant solutions. All patients had a history of miscarriage.

### 2.2. Wet Amount Preparation and Staining

After the vaginal discharge collection, the smear was prepared. Afterward, one or two drops of normal saline

solution were put on the prepared smear with a cover slip and were examined under a microscope. Gram stain kits (Hi-media, India) were used to differentiate between the shapes of the bacterial isolates. The wet mount technique was then utilized used for the detection of *Trichomonas vaginalis* and the Aerobic BV.

### 2.3. Biochemical Examination

All the samples were used for smear gram staining and were cultured on blood agar, chocolate agar, as well as MacConkey agar. Biochemical examinations performed for the suspicious cultures included the Urease test, Oxidase test, Coagulase test, and Catalase test (4).

### 2.4. Vitek-2 Diagnostic System of Bacterial Vaginosis

The Vitek-2 device (Biomérieux Diagnostics, France) was used to diagnose bacteria isolates from culture media, according to Pincus (5).

The Immunoglobulin M (IgM) and Immunoglobulin G (IgG) were evaluated using VIDAS CMV assay kits (Biomérieux Diagnostics, France). The determination of IgG avidity was conducted with a VIDAS CMV (IgG) Avidity II commercial kit (Biomérieux Diagnostics, France). Avidity examines the force of connection between an antibody-dependent and multiple parity antigens, which was determined via two VIDAS CMV IgG assays.

### 2.5. Statistical Analysis

The SPSS software (version 23) was used for the statistical analysis, and a *P*-value of 0.05 was considered statistically significant.

## 3. Results and Discussion

In the present study, the participants' age ranged from 14 to 45 years old, and they were divided into four age groups (Table 1). A high rate of miscarriage 48 (42.5%) was observed in women aged 24-34 years, which was considered a high incidence rate. The high incidence percentage occurred due to a high level of estrogen hormone, an elevated level of pH, and changes in the secretion of glycogen in women having a vaginal

miscarriage, which led to changes in the growth of the pathogen miscarriage. These were described in a previous study conducted by Magnus, Wilcox (6) who investigated the role of the marital age of pregnancy in the risk of miscarriage. The findings of their study revealed a miscarriage rate of 10.8% in the age group ranging from 30-34 years, which was in contrast with the findings of the present study.

**Table 1.** Age of miscarriage in women

Age group	Frequency	%
14-24	34	(30.10)
24-34	48	(42.5)
35-44	24	(21.2)
≥45	7	(6.2)
Total	113	100%

The recorded data showed that the majority of women who had a miscarriage had low levels of education. This finding is consistent with the findings of a study conducted by Norsker, Espenhain (7) indicating that women with low levels of education have a higher risk of spontaneous abortion. The study conducted by Nohr EA, Frydenberg M (8) was based on a large population and a large number of spontaneous abortions. The results of their study showed that women with less than 10 years of education had a higher chance of spontaneous abortion, compared to women with more than 12 years of education. Women’s level of education is one of the most important investments that empowers them to postpone marriage and childbirth. It also provides them with enough information to make informed decisions about using contemporary contraception. Many governments are currently funding women’s education to promote economic growth, smaller family sizes, better child health, and women’s sexual reproductive health (modern family planning) (9). A lack of health-conscious initiatives is the most common cause of miscarriage among women with primary education and poor hygiene whereas mothers who are educated are more concerned about their health, as shown in table 2.

**Table 2.** Education associated with miscarriage

Education	Once	Twice	More than twice	P-value
Primary	20(40)	31(62)	5(38.5)	0.03*
Secondary	16(32)	7(14)	6(46.2)	
Higher	14(50)	12(24)	2(15.4)	
Total	50(100%)	50(100%)	13(100%)	113(100%)

( $P \leq 0.05$ )\*

Table 3 presents the effect of the correlation between the type of contraceptive and miscarriage in recurrent miscarriages. The results of the current study were in contrast with those of a study conducted by Eschenbach, Patton (10) recording that the use of oral contraceptives did not have any significant effect on the normal flora of the genital tract. In a study conducted by Gupta, Hillier (11), it was found that oral contraceptives altered the vaginal flora and increased the count of *Escherichia coli* (*E. coli*) five times, which initiates infection and leads to miscarriage. The results of a study conducted by Fiscina, Oster (12), as well as Al-Kaisi (13), showed that an association is made between the use of an intrauterine contraceptive device and the normal vaginal flora from copper toxicity to the vagina and the change of bacterial flora.

**Table 3.** Vaginal discharge consistency associated with miscarriage

Miscarriage	Consistency of discharge			Total	P-value
	Thin%	Thick%	Crud%		
Once	7(14)	10(20)	33(66)	50(100%)	0.15*
Twice	12(24)	15(30)	23(46)	50(100%)	
More than twice	2(15.4)	3(23.1)	8(61.5)	13(100%)	
Total				113(100%)	

### 3.1. Aerobic Bacterial Vaginosis Associated with Miscarriage

Recurrent abortion was linked to BV in women (14). In the present study, the rate of BV was 103 (93.7%), as shown in table 4. The results of this study are in agreement with the findings of Xia, Li (14) indicating a significant relationship between BV and recurrent spontaneous abortion. In another study, Llahi-

Camp, Rai (15) found that women with a history of at least one late miscarriage had a significantly greater prevalence ( $P=0.001$ ) of BV (21%). The total microorganisms recovered from women's miscarriages include *Neisseria* spp. (3.6%), *Acinetobacter* spp. (0.9%), *Bacillus* spp. (23.6%), *E. coli* (22.7%), *Staphylococcus Sciuri* (0.9%), *Micrococcus Luteus* (4.5%), *Enterobacter Cloacae* (0.9%), *Kocuria Kristinae* (3.6%), *Staphylococcus Haemolyticus* (6.3%), *Enterococcus Faecalis* (8.1%), *Staphylococcus Aureus* (9.09%), *Burkholderia Cepacian* (2.7%), *Pseudomonas* spp. (1.8%), *Staphylococcus Warneri* (1.8%), *Enterococcus Faecium* (1.8%), *Streptococcus* spp. (2.7%), and *Proteus* spp. (0.9%). Several authors have suggested that an abnormal genital tract bacterial colonization can lead to in utero inflammatory and that BV in pregnant women can result in complications, including premature rupture of membranes and preterm delivery, respiratory distress syndrome, or necrotizing enterocolitis, such as *Enterococcus faecalis*, in preterm infants. *E. coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* are common microbes found in inflammatory vaginitis (16). This study discovered a significant incidence of *E. coli*, which can colonize the vagina and contribute to the replacement of the native microflora by removing *Lactobacilli* spp. When compared to *Enterococcus* spp., aerobic vaginosis is less frequently identified, yet it might cause frequent miscarriages, chorioamnionitis, and premature birth during pregnancy (17). However, the findings of this study revealed that *Klebsiella* spp. causes more infection [26 (23.6%)] in women having a miscarriage, compared to other bacteria.

**Table 4.** Aerobic bacterial vaginosis isolates in women having a miscarriage

NO	Aerobic bacterial vaginosis	Frequency	%
1	<i>Neisseria</i> spp.	4	3.6
2	<i>Acinetobacter</i> species	1	0.9
3	<i>Bacillus</i> species	4	3.6
4	<i>Klebsiella</i> species	26	23.6
5	<i>Escherichia Coli</i>	25	22.7
6	<i>Staphylococcus Sciuri</i>	1	0.9
7	<i>Micrococcus Luteus</i>	5	4.5
8	<i>Enterobacter Cloacae</i>	1	0.9
9	<i>Kocuria Kristinae</i>	4	23.6
10	<i>Staphylococcus Haemolyticus</i>	7	6.3
11	<i>Enterococcus Faecalis</i>	9	8.1
12	<i>Staphylococcus Aureus</i>	10	9.09
13	<i>Burkholderia Cepacia</i>	3	2.7
14	<i>Pseudomonas</i> species	2	1.8
15	<i>Staphylococcus Warneri</i>	2	1.8
16	<i>Enterococcus Faecium</i>	2	2.7
17	<i>Streptococcus</i> species	3	2.7
18	<i>Proteus</i> species	1	0.9
Total		110	100%

The results showed that aerobic BV leads to abortion rates of 28.6% (once a time) and 71.4% (twice a time) in the studied population (Table 5). It was also indicated that 50% of the studied population, who were infected with CMV or *Trichomonas vaginalis*, experienced abortion once and the other 50% experienced it twice. Of the 102 samples infected with *Lactobacillus* spp., 45.17% experienced abortion once and 42.2% experienced miscarriage two times (Table 5). The findings agree with the results of a previous study by Natacha Teissier, Fallet-Bianco (18) recording a high rate of premature delivery or abortion at 21 weeks from pregnancy. Razzak, Al-Charrakh (19) reported that the highest rates of BV were caused by gram-negative bacteria (16.2%).

**Table 5.** Pathogen associated with miscarriage

Pathogen	Once	Twice	More than twice	Total	P-value
Aerobic bacterial vaginosis	2(28.6%)	5(71.4%)	0	7(100%)	
Cytomegalovirus	1(50%)	1(50%)	0	2(100%)	
<i>Trichomonas vaginalis</i>	1(50%)	1(50%)	0	2(100%)	
<i>Lactobacillus</i> species	46(45.17)	43(42.2)	13(12.7)	102(100%)	0.35*
Total	50(44.2)	50(44.2)	13(11.5)	113(100%)	

( $P \leq 0.05$ )\*

### Authors' Contribution

Study concept and design: H. D. H.

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Acquisition of data: Z. A. D. A.

Analysis and interpretation of data: H. D. H.

Drafting of the manuscript: Z. A. D. A.

Critical revision of the manuscript for important intellectual content: H. D. H.

Statistical analysis: H. D. H.

Administrative, technical, and material support: H. D. H. and Z. A. D. A.

### Ethics

The study design was approved by the ethics committee of University of Misan, Amarah, Iraq.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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