Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
C M B Association Publisher

Vaginal Microbiota Profile in first-trimester miscarriages cases

Saman Rafeeq Abdullah[1,2*], Sarmad Raheem Kareem[3], Ayad H. Hasan[4,5]

[1] Department of Community Health, Koya Technical Institutes, Erbil Polytechnic University, Erbil, Iraq
[2] Department of Biology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq
[3] Faculty of Medicine, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq
[4] Department of Medical Microbiology, Faculty of Science and Health, Koya University, Koya KOY45, Kurdistan Region - F.R. Iraq
[5] Department of Medical Laboratory Science, College of Science, Cihan University-Erbil, Kurdistan Region, Iraq

ARTICLE INFO

Original paper

Article history:
Received: January 23, 2023
Accepted: June 08, 2023
Published: August 31, 2023

Keywords:

First-trimester miscarriage, vaginal, bacterial vaginosis, bacteria

ABSTRACT

Due to its rising prevalence, first-trimester miscarriage is getting more attention. Abortion etiology and pathology, especially in non-pathological cases, are unknown. In this study, 435 vaginal swabs were collected from aborted women in Maternity Teaching Hospitals in Erbil and Shahid Dr. Khalid Hospital in Koya. We characterized the vaginal microbiota diversity and composition in first-trimester abortion and investigated the association between bacterial vaginosis and abortion before 12 weeks. Cultural, morphological, and biochemical characteristics for each bacterial genus were discovered, and VITEK-2 system was used to identify isolated bacteria. Samples from each type of bacteria were selected for sequencing utilizing *16 rRNA* genes examining *V4–V8* region for bacterial profiles. Bacterial vaginitis was found in 412 (94.7%) first-trimester miscarriages. Six Gram-positive and four Gram-negative bacteria were found in these 412 samples. Microorganism distribution varied Gram-positive bacteria *Staphylococcus aureus* (86) 20.87%, *Enterococcus faecalis* (31) 7.52%, *Gardnerella vaginalis* (24) 5.83%, *Streptococcus agalactia* (21) 5.1%, *Lactobacillus equicursoris* (14) 3.4% and *Staphylococcus haemolyticus* (12) 2.91%. Gram-negative bacteria including *E. coli* (107) 25.97%, *Klebsiella pneumonia* (76) 18.45%, *Pseudomonas aeruginosa* (29) 7.04% and *Proteus mirabilis* (12) 2.91%. *Staphylococcus aureus* had the highest rate of isolation at (86) 20.87%, while *Lactobacillus equicursoris* had the lowest rate at (14) 3.4%. Overall, the rate of isolation for Gram-negative bacteria (224) was 54.4%, while the rate for Gram-positive bacteria (188) was 45.6%.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.8.2
Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

A first-trimester miscarriage occurs before the fetus is viable, 15.3% of diagnosed pregnancies experience miscarriages, one of the most common early pregnancy complications (1). Over 80% of miscarriages occur in the first 12 weeks (2). Miscarriages have become a global reproductive health issue, affecting physical, mental, and economic wellbeing (3). Human development and immunity depend on the microbiome. Female reproductive system health depends on maintaining a healthy balance in the vagina's numerous microbial communities (4). In reproductive-aged women, vaginal microbiome dysbiosis is connected to several illnesses, including reproductive issues. Premature birth (5), intrauterine adhesions (6), and infertility are reproductive diseases (7). However, few studies have examined the vaginal microbiome and miscarriage. Because the female genital tract (FGT) is suitable for pathogenic bacteria, several infections are common; it is populated by many helpful bacteria that do not cause illness until abnormal conditions are present. (8).

Pregnancy changes the vaginal microbiota and during pregnancy, dominated by *Lactobacillus spp*. (9). This shift may impede pathogen growth by secreting antimicrobial bacteriocins such as lactic acid, which keeps the pH low (10, 11). A disrupted vaginal environment can cause pregnancy difficulties, including premature birth (12). Bacterial vaginosis (BV) is a vaginal flora imbalance of pathogenic mixed flora of aerobic, anaerobic, and microaerophilic species proliferate and lactobacilli levels drop (13, 14). Inflammation of the vaginal epithelium and aberrant vaginal microbiota with aerobic and enteric bacteria like *E. coli*, *Klebsiella spp.* (15), *Gardnerella vaginalis*, and (16). *Enterococci, group B streptococci* (17). *Staphylococcus aureus, Klebsiella pneumonia*, *Streptococcus spp.*, and *Proteus spp.* were the most prevalent vaginal microbes (18). However, BV are both vaginal dysbioses with fewer lactobacilli (19). Conventional microbiological methods are limited in BV patient evaluation. Culture-based identification of single "marker" species is insensitive and specific because the sickness disrupts the normal vaginal microbiome (21). Because many BV-associated organisms are obligate anaerobes, traditional culture methods cannot recover them. Culture cannot accurately assess vaginal microbiota (22). Culture-dependent approaches for BV identification may be hindered by the non-cultivability of many bacterial members, which may change the profile of BV causative agents and their roles, culture-dependent BV identification approaches remain the gold standard notwithstanding these limitations (23).

* Corresponding author. Email: saman.abdullah@epu.edu.iq

Recent cultivation-independent molecular-based methods, such as broad-range bacterial *16S rRNA* PCR, revealed BV's genuine microbial diversity (24). Fortunately, *16S rRNA* gene sequencing and other microbial detection methods have improved, allowing fresh knowledge to accumulate quickly (25). *16S rRNA* sequencing has helped clinical microbiology labs identify bacterial isolates and new species. *16S rRNA* sequencing can identify bacteria with abnormal phenotypic profiles, rare bacteria, slow-growing bacteria, bacteria that cannot be cultivated, and culture-negative diseases. It has illuminated infectious disease etiologies and helped clinicians choose antibiotics, therapy length, and infection control methods. It also illuminated infectious disease etiologies (26). These approaches showed that BV was linked to several undiscovered bacteria (27).

Materials and Methods

Sample collection

This study includes 412 vaginal swabs collected from miscarried women in Maternity Teaching Hospitals in Erbil city and Shahid Dr. Khalid Hospital in Koya city from 1 December 2020 to June 2022. The women were chosen based on their pregnant weeks. The vaginal swab cultured on TSA blood agar using the streaking method and then they were incubated at 37 °C for 24 hours for identification by conventional methods and confirmed by VITEK 2 compact systems and molecular study (28, 29).

Characteristics of Cultural Isolation

Four different plates media were used in this study, the sample was taken into Tryptic Soy Agar (TSA) for both aerobic and anaerobic growth, then a single colony from the growth transfer to plates containing 5% sheep blood agar, MacConkey and chocolate agar, while blood agar and MacConky agar were used for the aerobic condition, Blood agar and Chocolate agar used for the anaerobic condition using candle Jar, and all cultures were incubated at 37 ℃ for 18-24 hours. Sub-culture onto the differential selective medium was achieved by inoculating a single colony into the plate by quadrant method The plates were examined for growth after 24 hours of incubation. In case of no bacterial growth, the plates were further incubated for 24 hours more. Then, after 48 hours the specimens with no growth, they were reported as negative, and finally the result of bacterial identification was recorded.

The identification at the species level depends on Grams staining, culture characteristics: colonies of bacteria, morphological characteristics viewed the bacteria at 1000X under the light microscope, changed the exhibition on the media like hemolysis, lactose fermentation, or non-lactose fermentation and motility. Conventional biochemical tests comprise catalase test, coagulase test (slide method), growth on mannitol salt agar, and hemolytic activity on blood agar plate, for gram-positive bacteria. On the other hand, oxidase test, urease test, triple sugar iron (TSI), indole production, methyl red, Voges-Proskauer, citrate utilization (IMViC) and hydrogen sulphide (H_2S) production were performed for Gram-negative bacteria. In addition, the identification was supported through both VITEK 2 compact system and the molecular approach. Gram-stain characteristics and relevant biochemical tests were carried out following the procedures described by (30). The bacterial isolates under study were identified according to references (31-33).

VITEK-2 Systems

The bacterial isolates were inoculated onto McConkey agar plates and then incubated overnight at 37°C. A single colony was then taken and suspended into solution. The turbidity of the bacterial suspension was adjusted with VITEK Densichek (bioMerieux) to match the McFarland 0.5 standard in 0.45% sodium chloride. Then the VITEK 2 ID-GN for Gram-negative bacteria card and ID-GP card for gram-positive bacteria, after that the bacterial suspension tubes were manually loaded into the VITEK-2 system. The following steps on the software were done according to the manufacturer’s instructions (Bio Merieux, France) (34).

Molecular method

DNA extraction

The total DNA of the bacterial genome was isolated by Genomic DNA Extraction Kit provided by (FAVORGEN, Taiwan. FABGK100) company, following the guidelines provided by the manufacturer. The quality of DNA was verified by electrophoresis in 1% agarose gel. Bands were visualized by a UV transilluminator Gel Documentation (InGenius LHR, Syngene, UK), and the quantity of DNA was determined using a nanodrop spectrophotometer (NanoVue plus, UK.). The extracted DNA was stored at -20 °C until further analysis. In the current study that provides a description of the primer pairs used in the research. The (Table 1) contains information on the specific primer sequences.

Application of Polymerase Chain Reaction

In this study, Gram-positive and Gram-negative bacteria were selected for analysis by PCR for detecting the 16S rRNA gene for each bacterium. The reagents required for the PCR reaction were mixed in a sterile (0.5ml) Eppendorf tube as shown in (Table 2).

The amplification program was run as follows:

-One cycle of 95°C for 2 minutes,

-Thirty cycles of 92°C for 1 minute, followed by 55 - 59°C for 1 minute depending on the type of the primer and 72°C for 1 minute,

Table 1. Primers used to amplify 16s rRNA, V4 and, V8 and their size.

Name	Primer	Sequence (5`- 3`)	Amplicon size base pair	Target gene
P5	Forward Reverse	TCATGGCTCAGATTGAACGC TCACCCCAGTCATGAATCACA	1476	*16S rRNA*
P6	Forward Reverse	CGCGGTAATACGGAGGGT GTCAGTCTTCGTCCAGGGG	229	*V4*
P7	Forward Reverse	CTGGAGGAAGGTGGGGATG TTCACCGTGGCATTCTGATC	204	*V8*

Table 2. PCR reaction mixture that was used for identification in this study (35, 36).

Component	Volume	Final conc.
Master mixture 2x	10 µl	2x
Forward primer 5 pmol/ µl	1 µl	0.1-1 µM
Reverse primer 5 pmol/ µl	1 µl	0.1-1 µM
DNA template	1 µl	25-50 ng
Nuclease-Free water	Volume adjusted to 20 _l using distilled water	

-One cycle of 72°C for 10 minutes.

The PCR products were separated electrophoresed on 1% of agarose (GeneDirex, USA) in Tris- Boric acid-EDTA at 80V for 1h, the expected size of the product 1476bp, were visualised using UV transilluminator Gel Documentation (InGenius LHR, Syngene, UK).

Gel Electrophoresis Analysis

A 3 µl of PCR reaction products were separated by electrophoresis on a 1% agarose gel including ethidium bromide (0.5 µg/ml) alongside a 100 bp DNA marker (GeneDirex, Marker 100bp) and ran in 1X TBE buffer at 80 V for one hour to verify that the targeted genes were amplified to the right size. Following running, the DNA amplicons were viewed and captured employing (UV Gel Imager SynGene).

Sequencing of the 16S rRNA Gene Amplicons

All types of bacterial samples were forwarded for sequencing, after the size of PCR products was confirmed, then sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation –Korea. The results were received by email and were then analyzed using Geneious software. The sequenced DNA was analyzed by the BLAST tool of the NCBI Gene Bank database which is available at the NCBI online at (https://www.ncbi.nlm.nih.gov/nuccore/?term=saman%20rafeeq%20abdullah).

Results

Sample Collection, Bacterial Isolation

A total of 435 vaginal swabs were collected from women who had experienced miscarriages in Maternity Teaching Hospitals located in Erbil city and Shahid Dr. Khalid Hospital in Koya city. The bacterial isolates obtained were identified by referring to Bergey's Manual of Systematic Bacteriology, which involved assessing their cultural, morphological, and biochemical characteristics. Additionally, the VITEK-2 system, a fully automated system widely used in clinical laboratories, was employed to support the identification process. The VITEK-2 system utilizes specific identification cards for both Gram-negative and Gram-positive bacteria, containing a panel of 48 tests for each bacterial type. Furthermore, for further analysis, three samples from each type of bacteria were selected and subjected to sequencing using the 16S rRNA genes.

Out of the 435 vaginal swabs collected, 412 samples (94.7%) exhibited positive culture results, indicating the presence of bacterial infection. On the other hand, 23 samples (5.3%) showed negative culture results. The findings of this study revealed a high prevalence of bacterial vaginitis in first-trimester miscarriages, as observed in 412 samples (94.7%). The analysis of these 412 samples identified several different types of bacteria, including six species of Gram-positive bacteria and four species of Gram-negative bacteria. A summary of the isolated bacteria can be found in Table 3.

The microorganisms showed variety in their percentages of distribution the Gram-positive bacteria *Staphylococcus aureus* (86) 20.87%, *Streptococcus agalactia* (21) 5.1%, *Gardnerella vaginalis* (24) 5.83%, *Staphylococcus haemolyticus* (12) 2.91 and *Enterococcus faecalis* (31) 7.52% and *Lactobacillus equicursoris* (14) 3.4%. Whereas the percentages of distribution for Gram-negative bacteria *E. coli* (107) 25.97%, *Klebsiella pneumonia* (76) 18.45%, *Proteus mirabilis* (12) 2.91% and *Pseudomonas aeruginosa* (29) 7.04%. Totally the number of isolation in Gram-negative bacteria (224) at 54.4% was greater than the number of Gram-positive bacteria (188) at 45.6%, The *Staphylococcus aureus* showed the highest number in isolation by (86) at 20.87%, while *Lactobacillus equicursoris* showed the lower number in isolation by (14) 3.4%.

The distribution of microorganisms in the samples revealed a variety of percentages. Among the Gram-positive bacteria, *Staphylococcus aureus* accounted for 20.87% (86 isolates), *Streptococcus agalactiae* for 5.1% (21 isolates), *Gardnerella vaginalis* for 5.83% (24 isolates), *Staphylococcus haemolyticus* for 2.91% (12 isolates), *Enterococcus faecalis* for 7.52% (31 isolates), and *Lactobacillus equicursoris* for 3.4% (14 isolates).

In contrast, the Gram-negative bacteria included *E. coli*

Table 3. The different types of bacteria isolated from BV.

Gram-positive bacteria	Number of isolates	Percentage %	Gram-negative bacteria	Number of isolates	Percentage %
Staphylococcus aureus	86	20.87	*E. coli*	107	25.97
streptococcus agalactiae	21	5.1	*Klebsiella pneumonia*	76	18.45
Gardnerella vaginalis	24	5.83	*Proteus mirabilis*	12	2.91
Staphylococcus haemolyticus	12	2.91	*Pseudomonas aeruginosa*	29	7.04
Enterococcus faecalis	31	7.52			
Lactobacillus equicursoris	14	3.4			
Total	188			224	

at 25.97% (107 isolates), *Klebsiella pneumonia* at 18.45% (76 isolates), *Proteus mirabilis* at 2.91% (12 isolates), and *Pseudomonas aeruginosa* at 7.04% (29 isolates). Overall, the number of Gram-negative isolates (224) accounted for 54.4% of the total, which was higher than the number of Gram-positive isolates (188) at 45.6%. Among the Gram-positive bacteria, *Staphylococcus aureus* had the highest number of isolates with 86 (20.87%), while *Lactobacillus equicursoris* had the lowest number with 14 isolates (3.4%).

Identification of isolates by VITEK2 system

The utilization of the VITEK 2 method allowed for precise identification of all types of isolates. The results obtained from the biochemical tests conducted on the bacterial isolates corresponded well with the results obtained from the VITEK 2 system, confirming their agreement.

Molecular detection

DNA extraction and Gel electrophoresis

Genomic DNA from the isolates was extracted using the Genomic DNA Extraction Kit provided by FAVORGEN, Taiwan (FABGK100). The concentration and purity of the extracted DNA were assessed using a Nanodrop instrument. Gel electrophoresis was performed using a 1% agarose gel and DNA Red safe dye, running at 80V for 1 hour. The results indicated that the DNA had good purity, with values ranging from 1.8 to 1.85, and the concentration ranged from 10 to 12 ng/µl. Additionally, the gel electrophoresis results exhibited clear and distinct bands of chromosomal DNA, as shown in Figure 1.

16S rRNA gene - PCR screening

This study employed 16S rRNA gene sequence-based analysis to validate the presence of bacterial DNA in 30 samples from miscarriage cases. This sequencing method is commonly used to study the vaginal microbiota and has enabled the detection of numerous aerobic and anaerobic species that were not previously identified through culture-based methods. In contrast, none of the control samples, including the negative extraction tubes, exhibited amplified bacterial DNA, indicating the absence of bacterial infection and the overall health of the fetuses. The study involved sequencing the 16S rRNA gene in a total of 30 miscarriage samples, generating deep V4 (229bp) and V8 (204bp) 16S rRNA gene profiles (as shown in Figures 2, 3 and 4) to gain further insights into the bacterial microbiome.

Figure 1. PCR amplified 16S rRNA of the isolates in 1% agarose gel. Lane M 100bp DNA ladder (GeneDirex). Lane N negative control that has been run without any DNA template, respectively. The bands are showing 1476bp of PCR amplicons. Lanes 1 to 10 contain PCR products from *Staphylococcus aureus , Staphylococcus haemolyticus , Lactobacillus equicursoris, Streptococcus agalactiae, Enterococcus faecalis, Gardnerella vaginalis, Klebsiella pneumonia, E. coli, Proteus mirabilis and Pseudomonas aeruginosa* respectively.

Figure 2. PCR amplified generate deep V8 (204bp) 16S rRNA of the isolates in 1% agarose gel. Lane M 100bp DNA ladder (GeneDirex). Lane N negative control that has been run without any DNA template, respectively. The bands are showing 1476bp of PCR amplicons. Lanes 1 to 10 contain PCR products from Staphylococcus aureus, Staphylococcus haemolyticus , Lactobacillus equicursoris, Streptococcus agalactiae, Enterococcus faecalis and Gardnerella vaginalis respectively.

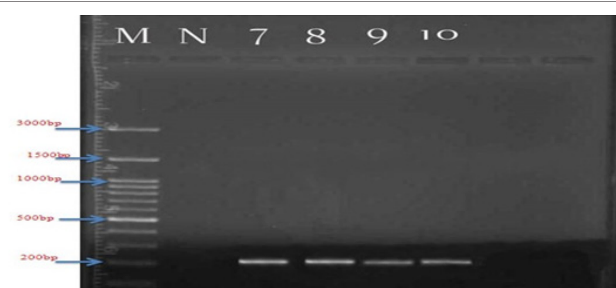

Figure 3. PCR amplified generate deep V8 (204bp) 16S rRNA of the isolates in 1% agarose gel. Lane M 100bp DNA ladder (GeneDirex). Lane N negative control that has been run without any DNA template, respectively. The bands are showing 1476bp of PCR amplicons. Lanes 1 to 10 contain PCR products from *Klebsiella pneumonia, E. coli, Proteus mirabilis* and *Pseudomonas aeruginosa* respectively.

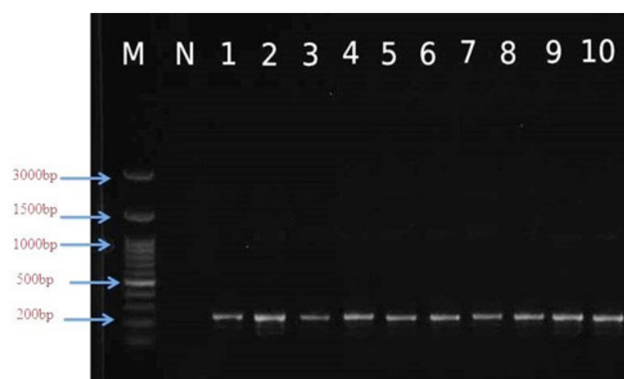

Figure 4. PCR amplified generate deep V4 (229bp) 16S rRNA of the isolates in 1% agarose gel. Lane M 100bp DNA ladder (GeneDirex). Lane N negative control that has been run without any DNA template, respectively. The bands are showing 1476bp of PCR amplicons. Lanes 1 to 10 contain PCR products from *Staphylococcus aureus, Staphylococcus haemolyticus, Lactobacillus equicursoris, Streptococcus agalactiae, Enterococcus faecalis, Gardnerella vaginalis, Klebsiella pneumonia, E. coli, Proteus mirabilis* and *Pseudomonas aeruginosa* respectively.

Sequence alignment

The gene sequence was analyzed using the Basic Local Alignment Search Tool (BLAST), which is a search tool available on the NCBI (National Center for Biotechnology

Information) website. BLAST applies the sequence alignment method to compare and align laboratory or query sequences with other biological sequences, enabling the identification of similarities with bacterial species.

Molecular Identification of bacteria

The amplified gene sequences, with sizes V8 (204bp) and V4 (229bp), were subjected to comparison using the BLAST program available on the GenBank database (http://blast.ncbi.nlm.nih.gov/). This comparison aimed to match our sequences with existing sequences of bacterial species stored in the database. The BLAST results revealed that there were 10 different bacteria with a 100% identity match to our query sequences. These alignments indicate that our query sequences should be submitted to the NCBI GenBank, and the corresponding accession numbers are provided in Table 4.

Phylogenetic inferences

Analysis of the 16S rRNA nucleotide sequence using phylogenetic methods demonstrated that the 10 bacterial species investigated exhibited grouping patterns consistent with expectations. Based on the similarity of sequence divergence data and the constructed phylogeny, it was observed that species within the same genera were closely related to one another (Figure 5).

Discussion

In many cases, the reasons behind first-trimester miscarriage are unknown (39). However, one significant factor associated with vaginal disease is the disruption of the normal bacterial balance, leading to a condition called bacterial vaginosis (BV). BV is highly prevalent among women worldwide and is considered a major cause of vaginitis (40). Research indicates that a significant number of miscarriage samples were found to be infected with bacteria compared to control samples without infection (41). The current study further supports the notion that the vaginal microbiota community contains various microorganisms capable of causing BV. Through analyzing vaginal swabs, we identified different bacterial species, suggesting that bacterial infection could be a significant contributor to miscarriages in women (42).

The presence of a healthy vaginal flora is crucial as it helps maintain a balanced pH and provides protection against infections. However, when there is an excessive growth of bacteria, particularly in the vagina, it disrupts

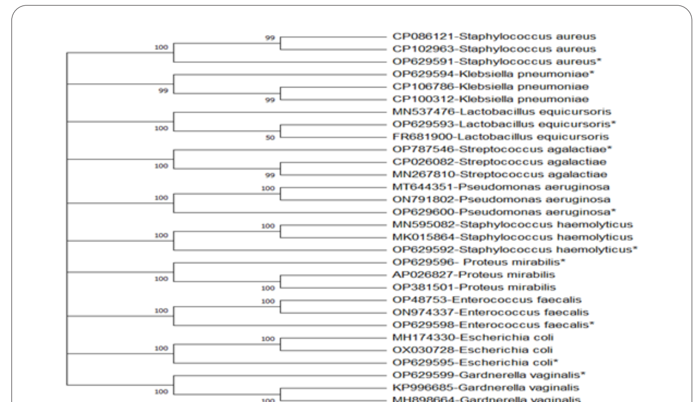

Figure 5. Phylogenic tree of bacteria sp. samples from Iraq: Kurdistan region (*). The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated partial 16S ribosomal rRNA gene were used as input data (38).

Table 4. Percentage distribution of samples bacterial species identification according to blast of GenBank NCBI of partial 16S rRNA.

Samples	Bacterial Identified	Accession Numbers	Query Cover %	Identic Number %	Accession Number of BLAST Identification	Country
1	*Staphylococcus aureus*	OP629591	100	100	CP086121	South Africa
			100	100	CP102963	Nepal
2	*Staphylococcus haemolyticus*	OP629592	100	100	MN595082	India
			100	100	MK015864	Netherlands
3	*Lactobacillus equicursoris*	OP629593	100	100	MN537476	Germany
			100	99.85	FR681900	France
4	*Klebsiella pneumoniae*	OP629594	100	100	CP106786	China
			100	100	CP100312	Italy
5	*E.a coli*	OP629595	100	100	MH174330	India
			100	100	OX030728	Spain
6	*Proteus mirabilis*	OP629595	100	99.85	AP026827	Viet Nam
			100	99.85	OP381501	Malaysia
7	*Streptococcus agalactiae*	OP787546	100	100	CP026082	China
			100	100	MN267810	United Arab Emirates
8	*Enterococcus faecalis*	OP629598	100	100	OP48753	Malaysia
			100	100	ON974337	China
9	*Gardnerella vaginalis*	OP629599	100	100	MH898664	Belgium
			100	100	KP996685	Portugal
10	*Pseudomonas aeruginosa*	OP629600	100	100	MT644351	Pakistan
			10	100	ON791802	China

the normal vaginal flora (43). Research has shown that a lower prevalence of *Lactobacillus spp.* (a type of bacteria) and a higher diversity of bacterial communities in the vagina are associated with an increased risk of first-trimester miscarriage. These changes in the vaginal bacterial composition occur prior to the diagnosis of miscarriage (44).

The isolation ratio of Lactobacillus spp. was found to be as low as 3.4%. Typically, a higher percentage of Lactobacillus spp. in the vaginal flora indicates a healthy vagina, whereas their presence in small quantities alongside a higher proportion of other pathogens indicates a pathogenic condition. *Lactobacillus spp.* play a crucial role in protecting against the invasion or excessive growth of pathogens through the production of hydrogen peroxide, bacteriocins, and lactic acid (45, 46). However, it is worth noting that certain strains of *Lactobacillus spp.* may be unable to produce certain defense factors when incubated under anaerobic conditions (47).

The incidence of *Gardnerella vaginalis* isolated in our study aligns with the rates reported in Basrah, where it was found to be 6.2% among women experiencing abortions (48). The prevalence of *Gardnerella vaginalis* in the current study is also consistent with findings from other developing countries such as South Africa (6.2%) (49) and Iraq (7.7%) (50). *Gardnerella vaginalis* is commonly observed as the most frequently identified pathogen in samples taken from individuals with vaginal infections (51).

Our findings regarding the detection of *Staphylococcus aureus* and *Enterococcus faecalis* are consistent with the results reported in reference (52), where the respective percentages were 20% and 10%. Similarly, our results align with previous studies (52-55) in terms of the detection of *Staphylococcus aureus* and *Proteus mirabilis*, with percentages of 30%, 0.7%, 20%, 5%, 23%, and 0.7%, and 19%, and 3% respectively. It has been reported that *Staphylococcus aureus* is one of the organisms known to cause septic abortion (56). Furthermore, certain strains of *Staphylococcus spp.* possess virulence factors that contribute to their increased pathogenicity (57).

In this study findings are in agreement with references (57, 58, 59, 60) regarding the detection percentages of *Enterococcus faecalis*, which were reported as 7.6%, 8.14%, 6.2%, and 5.89% respectively. Additionally, our results align with reference (55) in terms of detecting *E. coli*, *Klebsiella spp*., and *Staphylococcus aureus* at percentages of 27.27%, 13.64%, and 22.73% respectively. Previous studies (54, 55, 61) have also confirmed the detection of *E. coli* at percentages of 28%, 29.82%, and 30% respectively. In a study conducted in Nigeria, researcher (62) found an association between *E. coli* and miscarriage in certain cases. This finding is consistent with the results reported in previous studies (54 and 61) regarding the detection of *Klebsiella pneumoniae* at percentages of 18% and 24.57% respectively.

In a study conducted by researcher (52), the detection rate of *streptococcus spp.* was found to be 10%, which aligns with our own findings of 7.04%. Another study by researcher (63) identified an association between induced abortion and colonization of group B Streptococcus, also known as *Streptococcus agalactiae*. The presence of this species in the genital tract has been linked to abortion and suggests that colonization of *Streptococcus agalactiae* in the genitourinary tract may increase the risk of early-onset diseases (64). Moreover, the colonization of these species in pregnant women is a significant factor contributing to premature rupture of membranes, advanced miscarriage, premature birth, and other adverse pregnancy outcomes (65).

The detection of *Pseudomonas aeruginosa* at a rate of 7.04%, which is similar to the finding reported by reference (53) at 9%. *Pseudomonas spp.* is recognized as a significant opportunistic pathogen in the vagina, and even minor changes in the vaginal environment can lead to alterations in its virulence factors (66).

Regarding *Staphylococcus haemolyticus*, in this study found a prevalence of 2.18%, which is consistent with the findings reported in references (67, 68) where the rates were 1.6% and 3.7% respectively. It is noteworthy that *Staphylococcus haemolyticus* has been identified as an important causative agent of abortion (69). Furthermore, previous reports from Iraq (references 52 and 70) have isolated these bacteria from bacterial vaginosis (BV) in women.

Recent studies have demonstrated that pregnant women with bacterial vaginosis (BV) have an increased risk of spontaneous abortion, ranging from two to threefold higher compared to women without BV (71, 72). Furthermore, research (73) has shown that BV is frequently observed in pregnant women who have a history of spontaneous abortion. The first trimester has been identified as the period with the highest rate of abortion according to studies conducted in married women in Tikrit city (74, 75, 76).

Numerous studies have established a link between BV and premature delivery as well as miscarriage. Therefore, it is recommended that women with a history of preterm deliveries undergo early screening for BV during pregnancy and receive appropriate treatment in the first trimester to prevent abortion (73, 77).

According to researcher (78), the main pathogens commonly isolated from women with bacterial vaginosis (BV) include *Pseudomonas spp., Staphylococcus aureus*, Coagulase-Negative *Staphylococci* (CoNS), *Acinetobacter spp., Klebsiella spp., Citrobacter spp., Proteus spp., Enterobacter spp., Streptococcus agalactiae*, and *E. coli.* They further suggest that controlling these bacterial infections is crucial for reducing the risk of stillbirth, infertility, and abortion.

Studies have indicated that Gram-positive bacteria, as well as *Enterococcus faecalis*, can cause infections by impairing the immune system. These findings may suggest that unfavorable conditions in the genital tract of women who experience abortion lead to the activation of opportunistic bacteria, subsequently infecting the uterus of pregnant patients and contributing to abortion (52).

Several factors can influence the effectiveness of culture techniques, including sample size, the specific culture media used, inhibitory additives, bacterial quantity and viability in the samples, and the number of samples taken (79). In this study, biochemical tests such as VITEK®2 were employed to confirm positive samples. The use of VITEK®2 is essential in the medical field as it provides quick and accurate methods for correctly identifying the infectious agent. Automated systems, like VITEK®2, enable the rapid diagnosis of infectious pathogens, saving valuable time in the process (82).

In order to overcome the difficulties related to isolating and precisely identifying these bacteria, alternative

approaches need to be implemented, it is recommended to employ culture-independent methods in future studies (80). Culture-dependent methods are prone to misidentification, and thus culture-independent genetic approaches are now preferred (81).

The gold standard tool for profiling bacterial vaginosis (BV) is still the 16S rRNA gene sequencing. However, due to cost and time considerations, it is not recommended for routine diagnostics or rapid processing of samples (83).

Conclusion

Molecular approaches are more sensitive in diagnoses of BV than traditional testing in the case of vaginal swab samples, according to the results of a study that determined numerous different species of bacteria were found in abortion cases that occurred during the first trimester.

Acknowledgment

The authors are grateful for the help they received from the Science and Health Research Center at Koya University and the Department of Biology in the Faculty of Science and Health at Koya University and Koya technical institutes, Erbil Polytechnic University.

References

1. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, Brosens JJ, Brewin J, Ramhorst R, Lucas ES, McCoy RC. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. The Lancet. 2021 May 1;397(10285):1658-67.
2. Tsikouras P, Deftereou T, Anthoulaki X, Bothou A, Chalkidou A, Gaitatzi F, Tsirkas I, Bourazan AC, Bampageorgaka E, Stanulov G, Chatzimichael E. Abortions in first trimester pregnancy, management, treatment. InInduced Abortion and Spontaneous Early Pregnancy Loss-Focus on Management 2019 Jun 25. IntechOpen.
3. MacWilliams K, Hughes J, Aston M, Field S, Moffatt FW. Understanding the experience of miscarriage in the emergency department. Journal of Emergency Nursing. 2016 Nov 1;42(6):504-12.
4. Greenbaum S, Greenbaum G, Moran-Gilad J, Weintraub AY. Ecological dynamics of the vaginal microbiome in relation to health and disease. American Journal of Obstetrics and Gynecology. 2019 Apr 1;220(4):324-35.
5. Kumar M, Murugesan S, Singh P, Saadaoui M, Elhag DA, Terranegra A, Kabeer BS, Marr AK, Kino T, Brummaier T, McGready R. Vaginal microbiota and cytokine levels predict preterm delivery in Asian women. Frontiers in cellular and infection microbiology. 2021 Mar 4;11:639665.
6. Cheah FC, Lai CH, Tan GC, Swaminathan A, Wong KK, Wong YP, Tan TL. Intrauterine Gardnerella vaginalis infection results in fetal growth restriction and alveolar septal hypertrophy in a rabbit model. Frontiers in pediatrics. 2021 Jan 22;8:593802.
7. Tomaiuolo R, Veneruso I, Cariati F, D'Argenio V. Microbiota and human reproduction: the case of female infertility. High-throughput. 2020 May 3;9(2):12.
8. Khamees SS. Characterization of vaginal discharge among women complaining of genital tract infection. International Journal of Pharmacy & life sciences. 2012 Oct 1;3(10).
9. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, Raza S, Rosenbaum S, Van den Veyver I, Milosavljevic A, Gevers D. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PloS one. 2012 Jun 13;7(6):e36466.
10. McLEAN NW, Rosenstein IJ. Characterisation and selection of a Lactobacillus species to re-colonise the vagina of women with recurrent bacterial vaginosis. Journal of medical microbiology. 2000 Jun;49(6):543-52.
11. Martin Jr HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. Journal of Infectious Diseases. 1999 Dec 1;180(6):1863-8.
12. Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. Bmj. 1994 Jan 29;308(6924):295-8.
13. Ray CG, Ryan KJ, editors. Sherris medical microbiology. New York, NY, USA: McGraw-Hill Education/Medical; 2014.
14. Seth AR, Chaitra S, Vaishnavi S, GR SC. Prevalence of bacterial vaginosis in females in the reproductive age group in Kadur, Karnataka, India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2017 Nov 1;6(11):4863-6.
15. Donders GG, Bellen G, Grinceviciene S, Ruban K, Vieira-Baptista P. Aerobic vaginitis: no longer a stranger. Research in Microbiology. 2017 Nov 1;168(9-10):845-58.
16. Lamont RF, Sobel JD, Akins RA, Hassan SS, Chaiworapongsa T, Kusanovic JP, Romero R. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG: An International Journal of Obstetrics & Gynaecology. 2011 Apr;118(5):533-49.
17. Donati L, Di Vico A, Nucci M, Quagliozzi L, Spagnuolo T, Labianca A, Bracaglia M, Ianniello F, Caruso A, Paradisi G. Vaginal microbial flora and outcome of pregnancy. Archives of gynecology and obstetrics. 2010 Apr;281:589-600.
18. Khan JA, Iqbal Z, Ali L, Khan A. Prevalence and B-Lactamase Producing Bacterial Isolates in A Teaching Hospital in Peshawar, Pakistan: A four years study.. Pakistan Journal of Pharmaceutical Sciences. 2008 Oct 1;21(4).
19. Son KA, Kim M, Kim YM, Kim SH, Choi SJ, Oh SY, Roh CR, Kim JH. Prevalence of vaginal microorganisms among pregnant women according to trimester and association with preterm birth. Obstetrics & gynecology science. 2018 Jan 1;61(1):38-47.
20. Cartwright CP, Lembke BD, Ramachandran K, Body BA, Nye MB, Rivers CA, Schwebke JR. Development and validation of a semiquantitative, multitarget PCR assay for diagnosis of bacterial vaginosis. Journal of clinical microbiology. 2012 Jul;50(7):2321-9.
21. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM. Colloquium paper: vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America. 2011 Mar 3;108(Suppl 1):4680.
22. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, Galuppi M, Lamont RF, Chaemsaithong P, Miranda J, Chaiworapongsa T. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. 2014 Dec;2(1):1-9.
23. Foxman B, Wen A, Srinivasan U, Goldberg D, Marrs CF, Owen J, Wing DA, Misra D. Mycoplasma, bacterial vaginosis–associated bacteria BVAB3, race, and risk of preterm birth in a high-risk cohort. American journal of obstetrics and gynecology. 2014 Mar 1;210(3):226-e1.
24. Kameoka S, Motooka D, Watanabe S, Kubo R, Jung N, Midorikawa Y, Shinozaki NO, Sawai Y, Takeda AK, Nakamura S. Benchmark of 16S rRNA gene amplicon sequencing using Japanese gut microbiome data from the V1–V2 and V3–V4 primer sets. BMC genomics. 2021 Dec;22(1):1-0.
25. Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use

of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clinical Microbiology and Infection. 2008 Oct 1;14(10):908-34.

26. Shipitsyna E, Roos A, Datcu R, Hallén A, Fredlund H, Jensen JS, Engstrand L, Unemo M. Composition of the vaginal microbiota in women of reproductive age–sensitive and specific molecular diagnosis of bacterial vaginosis is possible?. PloS one. 2013 Apr 9;8(4):e60670.
27. VITEK® 2 Systems Product Information Manual. Durham (NC): bioMérieux, Inc; 2016.
28. Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB, editors. Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes. Springer Science & Business Media; 2011 Jan 28.
29. Harley, J.P. Laboratory Exercises in Microbiology. 10th ed. McGraw-Hill Higher Education. New York. 2016.
30. MacFaddin J F,Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore, 2000.
31. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. Cambridge: Cambridge University Press; 2004.
32. Forbes BA, Sahm DF, Weissfeld AS. Study Guide for Bailey and Scott's Diagnostic Microbiology-E-Book. Elsevier Health Sciences; 2016 Jun 30.
33. Jebur DM, Abood ZH. Isolation and Detection of Cronobacter sakazakii from Infant Dried Milk using PCR and RT-PCR techniques. Iraqi journal of biotechnology. 2018 Nov 12;17(2).
34. Anbazhagan D, Kathirvalu GG, Mansor M, Yan GS, Yusof MY, Sekaran SD. Multiplex polymerase chain reaction (PCR) assays for the detection of Enterobacteriaceae in clinical samples. Afr. J. Microbiol. Res. 2010 Jun 4;4(11):1186-91.
35. Turton, J.F., Woodford, N., Glover, J., Yarde, S., Kaufmann, M.E. and Pitt, T.L., Identification of Acinetobacter baumannii by detection of the bla OXA-51-like carbapenemase gene intrinsic to this species. Journal of clinical microbiology, 2006.44(8), pp.2974-2976.
36. Boone DR, Castenholz RW, Garrity GM, editors. Bergey's manual® of systematic bacteriology. Springer Science & Business Media; 2001.
37. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997 Sep 1;25(17):3389-402.
38. Suzumori N, Sugiura-Ogasawara M. Genetic factors as a cause of miscarriage. Current medicinal chemistry. 2010 Oct 1;17(29):3431-7.
39. Coleman JS, Gaydos CA. Molecular diagnosis of bacterial vaginosis: an update. Journal of clinical microbiology. 2018 Sep;56(9):e00342-18.
40. Allanson B, Jennings B, Jacques A, Charles AK, Keil AD, Dickinson JE. Infection and fetal loss in the mid-second trimester of pregnancy. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2010 Jun;50(3):221-5.
41. Ahmed S, Lutalo T, Wawer M, Serwadda D, Sewankambo NK, Nalugoda F, Makumbi F, Wabwire-Mangen F, Kiwanuka N, Kigozi G, Kiddugavu M. HIV incidence and sexually transmitted disease prevalence associated with condom use: a population study in Rakai, Uganda. Aids. 2001 Nov 9;15(16):2171-9.
42. Nigro G, Mazzocco M, Mattia E, Di Renzo GC, Carta G, Anceschi MM. Role of the infections in recurrent spontaneous abortion. The Journal of Maternal-Fetal & Neonatal Medicine. 2011 Aug 1;24(8):983-9.
43. Al-Memar M, Bobdiwala S, Fourie H, Mannino R, Lee YS, Smith A, Marchesi JR, Timmerman D, Bourne T, Bennett PR, MacIntyre DA. The association between vaginal bacterial composition and miscarriage: a nested case–control study. BJOG: An International Journal of Obstetrics & Gynaecology. 2020 Jan;127(2):264-74.
44. Motevaseli E, Shirzad M, Raoofian R, Hasheminasab SM, Hatami M, Dianatpour M, Modarressi MH. Differences in vaginal lactobacilli composition of Iranian healthy and bacterial vaginosis infected women: a comparative analysis of their cytotoxic effects with commercial vaginal probiotics. Iranian Red Crescent Medical Journal. 2013 Mar;15(3):199.
45. Doyle R, Gondwe A, Fan YM, Maleta K, Ashorn P, Klein N, Harris K. A Lactobacillus-deficient vaginal microbiota dominates postpartum women in rural Malawi. Applied and Environmental Microbiology. 2018 Mar 15;84(6):e02150-17.
46. Razzak MS, Al-Charrakh AH, Al-Greitty BH. Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginitis. North American Journal of Medical Sciences. 2011 Apr;3(4):185.
47. Mahdi NK, Al Hamdani MM. Sexually transmitted diseases among women with habitual abortion. EMHJ-Eastern Mediterranean Health Journal,1998. 4 (2), 343-349,
48. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. The American journal of medicine. 1983 Jan 1;74(1):14-22.
49. Al-Muk JM, Hasony HJ. Isolation of Gardnerella vaginalis from pregnant women with bacterial vaginosis in Basrah, Iraq. Bahrain Medical Bulletin. 2001 Sep;23(3):124-6.
50. Hashemi H, Shakibapour M, Fazeli H, Varshowsaz J, Aliyari Z, Darani HY. Molecular Study of Gardnerella vaginalis Isolated from Vaginal Discharge of Women Referring to Gynecology Clinics in Chaharmahal and Bakhtiari, Iran, in 2017. Archives of Clinical Infectious Diseases. 2021 Jun 30;16(2).
51. AL-Karbolii MM, Alwan MJ. Bacterial Isolation and Identification from Aborted Placenta of Women and Ewes and Goat in Al-Fallujag City. Plant Archives. 2020;20(1):125-31.
52. Abdulla, K.N., Shaker, S.R. and Fawzi, H.A., Study on bacterial vaginosis among a group of Iraqi women. Medical Science, 2020. 4(105), pp.3012-3016.
53. Bitew A, Abebaw Y, Bekele D, Mihret A. Prevalence of bacterial vaginosis and associated risk factors among women complaining of genital tract infection. International Journal of Microbiology. 2017 Oct;2017.
54. Asif H. A., Leqaa M. A., Mohammed J. M. and Ahmed N. L. , Microorganism Distribution that Causes Abortionin Females of Fallujah City. 2020.20(2).
55. Grudzinkas, J. G. Miscarriages, ectopic pregnancy and trophpoblastic disease. In Edmonds K. (Ed) Dewhurst Textbook of Obstetrics and Gynaecology for Post th Graduate students. 7th edition. London. Blackwell Science. 2012. 61-75.
56. AlSultany, Sh. J. and Jassim,Y.A. 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, (2016). 7 (3) Page No. 1530.
57. Ghasemi E, Mansouri S, Shahabinejad N. Vaginal colonization and susceptibility to antibiotics of enterococci during late pregnancy in Kerman City, Iran. Archives of Clinical Infectious Diseases. 2016 Oct 1;11(4).
58. Khdir KH. Molecular and Bacteriological Study of Enterococcus faecalis Isolated from Different Clinical Sources. Zanco Journal of Pure and Applied Sciences. 2020;32(2).
59. Daza R, Gutierrez J, Piédrola G. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. International journal of antimicrobial agents.

2001 Sep 1;18(3):211-5.

60. Al Sweih N, Jamal W, Rotimi VO. Spectrum and antibiotic resistance of uropathogens isolated from hospital and community patients with urinary tract infections in two large hospitals in Kuwait. Medical Principles and Practice. 2005;14(6):401-7.
61. Salih NI, Hassan MF. Evaluation the relation of TNF-alpha in aborted women who suffer from bacterial vaginitis. Medical Journal Of Tikrit University. 2018;24(2):95-103.
62. Okoroiwu .G. and Wachukwu C. Studies on the Role of Escherichia coli in Pregnancy Related Miscarriages (Spontaneous Abortions) In Upland Area of Rivers State of Nigeria.Am. J. PharmTech Res.; 2015.5(6): 386-394.
63. Chen Z, Wen G, Cao X, Li S, Wang X, Yao Z, Wu CA, Ye X. Group B streptococcus colonisation and associated risk factors among pregnant women: a hospital-based study and implications for primary care. International journal of clinical practice. 2019 May;73(5):e13276.
64. Colbourn T, Gilbert R. An overview of the natural history of early onset group B streptococcal disease in the UK. Early human development. 2007 Mar 1;83(3):149-56.
65. Li YP, Kuok CM, Lin SY, Hsieh WS, Shyu MK. Group B streptococcus antimicrobial resistance in neonates born to group B streptococcus-colonized mothers: Single-center survey. Journal of Obstetrics and Gynaecology Research. 2016 Nov;42(11):1471-5.
66. Puri N. Study on the incidence of urinary tract infection in diabetic patients and the prevalence of multidrug resistant strains among the bacterial pathogenic isolates (Doctoral dissertation, Department of Microbiology).2006.
67. Alwaily E, Khazaal R, Flaih M, Hussein K. Molecular Characterization of Staphylococcus Haemolyticus Isolated from Vaginitis and Some of Their Virulence Factors. InProceedings of 2nd International Multi-Disciplinary Conference Theme: Integrated Sciences and Technologies, IMDC-IST 2021, 7-9 September 2021, Sakarya, Turkey 2022 Jan 26.
68. Al-Wandawy AH, Zwain LA, Omer SA, Al-Wandawy AH. Investigation of vaginal bacteria in healthy and in women with genital infection. Ann. Trop. Med. 2020.
69. Obais, S.Y.A. and Alsultany, S.J., The Microbiological Study of Bacterial Vaginosis in Women with Recurrent Spontaneous Abortion in Babylon Province. Annals of the Romanian Society for Cell Biology, 2021.pp.4579-4591.
70. Razzak MS, Al-Charrakh AH, Al-Greitty BH. Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginitis. North American Journal of Medical Sciences. 2011 Apr;3(4):185.
71. Ralph SG, Rutherford AJ, Wilson J. Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. Bmj. 1999 Jul 24;319(7204):220-3.
72. Ugwumadu A, Manyonda I, Reid F, Hay P. Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial. The Lancet. 2003 Mar 22;361(9362):983-8.
73. Abdullateef RM, Ijaiya MA, Abayomi F, Adeniran AS, Idris H. Bacterial vaginosis: Prevalence and associated risk factors among non-pregnant women of reproductive age attending a Nigerian tertiary hospital. Malawi Medical Journal. 2017;29(4):290-3.
74. Ra'ad AZ, Ahmed TH, Najim WS. Diagnosis of vaginitis in married women by microbiogical and molecular methods in Tikrit City. Tikrit Medical Journal. 2015 Jun 1;20(1).
75. Paradisi R, Porcu E, Venturoli S, Maldini-Casadei M, Boni P. Maternal serum levels of pro-inflammatory cytokines in missed and threatened abortion. American Journal of Reproductive Immunology. 2003 Oct;50(4):302-8.
76. Abdullah GA, Mahdi NK. The role of cytokines among women with spontaneous miscarriage. Medical Journal of Islamic World Academy of Sciences. 2013;21(3):119-24.
77. Bretelle F, Rozenberg P, Pascal A, Favre R, Bohec C, Loundou A, Senat MV, Aissi G, Lesavre N, Brunet J, Heckenroth H. High Atopobium vaginae and Gardnerella vaginalis vaginal loads are associated with preterm birth. Clinical Infectious Diseases. 2015 Mar 15;60(6):860-7.
78. Castellano Filho DS, Diniz CG, Silva VL. Bacterial vaginosis: clinical, epidemiologic and microbiological features. HU Revista. 2010;36(3).
79. Collee, J.,Fraser, A.,Marmion, B. and Simons A. Mackie and McCartney's Practical Medical Microbiology. Fourteenth Edition. Churchill livingistone, U.S.A., 1996.p:561
80. Martínez-Peña MD, Castro-Escarpulli G, Aguilera-Arreola MG. Lactobacillus species isolated from vaginal secretions of healthy and bacterial vaginosis-intermediate Mexican women: a prospective study. BMC infectious diseases. 2013 Dec;13:1-9.
81. Petricevic L, Domig KJ, Nierscher FJ, Krondorfer I, Janitschek C, Kneifel W, Kiss H. Characterisation of the oral, vaginal and rectal Lactobacillus flora in healthy pregnant and postmenopausal women. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2012 Jan 1;160(1):93-9.
82. Moehario LH, Boestami HP, Edbert D, Tjoa E, Robertus T. Automation for the identification of Pseudomonas aeruginosa: Comparison of TDR-300B, VITEK® 2 and VITEK®-MS. bioRxiv. 2019 Jan 4:510107.
83. Singer M, Koedooder R, Bos MP, Poort L, Schoenmakers S, Savelkoul PH, Laven JS, de Jonge JD, Morré SA, Budding AE. The profiling of microbiota in vaginal swab samples using 16S rRNA gene sequencing and IS-pro analysis. BMC microbiology. 2021 Dec;21(1):1-0.