Vaginal Microbiota Profile in first-trimester miscarriages cases

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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 vaginosis 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%.

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 sp. (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).
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 °C 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 (H₂S) 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.

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 bacterial isolates were inoculated onto McConkey 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 (H₂S) 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).

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer</th>
<th>Sequence (5’ - 3’)</th>
<th>Amplicon size base pair</th>
<th>Target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5</td>
<td>Forward</td>
<td>TCAACCCAGCTCATGTTCA</td>
<td>1476</td>
<td>16S rRNA</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTGGTGTGTTGAAGCCGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>Forward</td>
<td>CGGCGGTTAACCGAGGGG</td>
<td>229</td>
<td>V4</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GTCAGTCTTCGGTCCAGGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>Forward</td>
<td>CTGAGGGAAGGTGGGGATG</td>
<td>204</td>
<td>V8</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TTCACCCGTTCCATTCTGATC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
-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 agalactiae* (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*...
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.

**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

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bacterial Identified</th>
<th>Accession Numbers</th>
<th>Query Cover %</th>
<th>Identity Number %</th>
<th>Accession Number of BLAST Identification</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>OP629591</td>
<td>100</td>
<td>100</td>
<td>CP086121</td>
<td>South Africa</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus haemolyticus</em></td>
<td>OP629592</td>
<td>100</td>
<td>100</td>
<td>CP102963</td>
<td>Nepal</td>
</tr>
<tr>
<td>3</td>
<td><em>Lactobacillus equicursoris</em></td>
<td>OP629593</td>
<td>100</td>
<td>100</td>
<td>MN595082</td>
<td>India</td>
</tr>
<tr>
<td>4</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>OP629594</td>
<td>100</td>
<td>100</td>
<td>MK015864</td>
<td>Netherlands</td>
</tr>
<tr>
<td>5</td>
<td><em>E. coli</em></td>
<td>OP629595</td>
<td>100</td>
<td>100</td>
<td>MN537476</td>
<td>Germany</td>
</tr>
<tr>
<td>6</td>
<td><em>Proteus mirabilis</em></td>
<td>OP629595</td>
<td>100</td>
<td>99.85</td>
<td>FR681900</td>
<td>France</td>
</tr>
<tr>
<td>7</td>
<td><em>Streptococcus agalactiae</em></td>
<td>OP787546</td>
<td>100</td>
<td>100</td>
<td>CP100312</td>
<td>China</td>
</tr>
<tr>
<td>8</td>
<td><em>Enterococcus faecalis</em></td>
<td>OP629598</td>
<td>100</td>
<td>100</td>
<td>CP106786</td>
<td>Italy</td>
</tr>
<tr>
<td>9</td>
<td><em>Gardnerella vaginalis</em></td>
<td>OP629599</td>
<td>100</td>
<td>99.85</td>
<td>CP026082</td>
<td>China</td>
</tr>
<tr>
<td>10</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>OP629600</td>
<td>100</td>
<td>100</td>
<td>MN267810</td>
<td>United Arab Emirates</td>
</tr>
</tbody>
</table>

![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).](image-url)
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 27.2%, 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.

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