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Screening For Multidrug-Resistant Staphylococcus Spp. Bacteria Causing Bacterial Vaginosis in Women: Penyaringan Bakteri Staphylococcus spp. yang Resisten terhadap Banyak Obat yang Menyebabkan Vaginosis Bakteri pada Wanita

Penyaringan Bakteri Staphylococcus spp. yang Resisten terhadap Banyak Obat yang Menyebabkan Vaginosis Bakteri pada Wanita

Zainab A. Fadhil, zainab.a.fadhil@uodiyala.edu.iq, ()

Department of Biology, College of Education for Pure Science, Diyala University/Iraq, Iraq

(1) Corresponding author

Abstract

Background: Bacterial vaginosis (BV) is a common vaginal condition characterized by a shift from lactobacilli dominance to opportunistic bacteria. **Specific Background:** Recent observations indicate the increasing role of *Staphylococcus spp.*, particularly multidrug-resistant strains, in persistent or recurrent BV. **Knowledge Gap:** Limited evidence exists regarding species-level identification and resistance profiles of *Staphylococcus spp.* in BV cases in Iraq. **Aim:** This study aimed to identify the bacterial species associated with BV and determine the antimicrobial susceptibility of the most resistant isolates. **Results:** Among 50 clinical samples, 20 yielded bacterial growth, dominated by Gram-positive isolates (65%), especially *Staphylococcus haemolyticus* (40%), *S. aureus* (15%), and *S. epidermidis* (10%). Gram-negative isolates included *E. coli* (20%) and *Klebsiella pneumoniae* (10%). *Gardnerella vaginalis* was detected in 5%. Antibiotic testing of 16 isolates showed high multidrug resistance, with *S. haemolyticus* exhibiting resistance to OFX, CRO, AMC, CTX, EM, CFM, and NA. **Novelty:** This study provides the first localized profiling of MDR *Staphylococcus spp.* in BV using VITEK-2 confirmation. **Implications:** Findings highlight the need for routine species identification, antimicrobial stewardship, and region-specific treatment guidelines to prevent rising resistance and recurrence.

Highlights
• Dominance of MDR *Staphylococcus spp.* in BV
• High resistance to β-lactams and macrolides
• Importance of routine screening and antibiotic stewardship

Keywords: Bacterial Vaginosis, Staphylococcus Haemolyticus, Multidrug Resistance, VITEK-2, Vaginal Microbiota

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Introduction

One of the most prevalent illnesses among women of reproductive age is bacterial vaginosis (BV), which raises the risk of premature birth infections and pelvic inflammatory disease (PID). which are the second leading cause of neonatal death worldwide [1]. Numerous studies highlight the importance of normal vaginal flora. The presence of vaginal microorganisms, predominantly Lactobacillus species, plays a crucial role in regulating vaginal health and preventing diseases.[2] A decrease in Lactobacillus and an increase in the number or type of facultative and anaerobic bacteria alter the vaginal bacterial balance, leading to an increase in pathogenic vaginal bacteria and causing BV. This, in turn, increases vaginal pH, which has been linked to increased susceptibility to and transmission of sexually transmitted infections [3]. Lactobacillus regulates the acidic environment of the vagina by producing abundant lactic acid, which acidifies the vagina. A pH <4.5 effectively contributes to protecting the vagina from pathogenic bacterial and viral infections [4].Hormonal changes in the vagina play a significant role in altering the natural vaginal flora, leading to inflammation. Normal estrogen levels are essential for maintaining vaginal balance and resistance to bacterial infections, as this hormone stimulates and activates the growth and integrity of the vaginal epithelium [5].

Numerous studies have established that various types of bacteria cause bacterial vaginosis, a polymicrobial infection. There are numerous causes of female reproductive tract infections (particularly vaginal and cervical infections), such as bacteria. [6]. These constitute (40-50%) of cases, with yeasts accounting for (30-40%), in addition to parasitic and viral infections. Vaginitis occurs in 20-40% of cases as a result of mixed infections caused by several organisms simultaneously [7].

The primary line of therapy for bacterial vaginosis is antibiotics. Intravaginal clindamycin and oral or intravaginal metronidazole are suggested treatment plans. [8]. These treatments have similar short-term efficacy against the illness. Within six to twelve months after finishing antibiotic therapy, bacterial vaginosis recurs in 50% to 80% of women. Antimicrobial resistance, biofilm development, reinfection through sexual partners, and the inability to restore ideal vaginal flora are all hypothesised causes of this therapeutic failure. [9]. Inaccurate diagnosis of bacterial vaginosis in women and premature initiation of treatment without prior drug sensitivity testing and laboratory examinations can lead to the emergence of antibiotic-resistant bacterial strains. Furthermore, the indiscriminate and excessive use of antibiotics has contributed to the development of resistant bacterial strains, and this resistance often becomes widespread [10]. Antimicrobial resistance may be influenced by biofilm, which is more commonly seen in people with recurrent bacterial vaginosis than in healthy people or those who have only had one episode. Even if bacterial vaginosis is successfully treated with antibiotics, the bacterial biofilm decreases the penetration of antimicrobials. Bacterial vaginosis (BV) requires a variety of treatment and preventative approaches since the biofilm is clinically persistent.[11]. Controlling pH, disrupting biofilms, and adhering to dietary changes, hormonal contraceptives, and condom use are some of these tactics. [12].

Materials and Methods

2.1 Sample Culture

In this study, (50) vaginal discharge samples were collected from pregnant women (35) and non-pregnant women (15), for the period from February 2023 to March 2023, from women arriving at Al-Batoul Teaching Hospital in Diyala Governorate and Al-Khansaa Teaching Hospital in Mosul. These samples included women aged 20-50 years, pregnant and non-pregnant, who had clinical symptoms associated with bacterial vaginosis (BV) and were transferred to the laboratory.

2.2 Diagnosis of Bacterial Isolates

The bacterial isolates obtained were diagnosed according to the following criteria [13].

2.2.1 Morphological Identification

The morphological characteristics of the bacterial isolates were studied on blood agar and MacConkey agar, including colony size, colour, consistency, margins, and other properties [14].

2.2.2 Microscopic Diagnosis

Bacterial isolates were diagnosed using Gram staining. Several colonies growing on blood agar or MacConkey agar were placed on a clean glass slide containing a drop of normal saline. The slide was spread on the slide and allowed to dry. It was then heat-fixed by rapidly passing it over a flame two or three times. Finally, it was stained with Gram stain and examined under a microscope to observe the morphology, color, cell aggregation, and staining pattern [15].

2.2.3 Biochemical Diagnosis

Before biochemical diagnosis, bacteria were activated on blood agar. Diagnostic tests, as described in Forbes et al. (2007) [13]. were used to identify bacterial isolates at the species level, as follows:

2 .2.3.1 Catalase Test

By turning hydrogen peroxide into water and oxygen gas, this test was used to determine if bacterial isolates were capable of producing the catalase enzyme. A tiny amount of the 18–24-hour-old bacterial growth was transferred onto the surface of

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a dry, clean glass slide in order to conduct this test. Hydrogen peroxide (H2O2) was added in a few drops. A positive result, or the release of oxygen gas, is shown by the production of air bubbles on the glass slide's surface. This shows that the isolates are capable of producing the catalase enzyme. (14).

2.2.3.2 Oxidase Test

This test was used to detect the ability of bacterial isolates to produce the oxidase enzyme. Filter paper was saturated with several drops of oxidase reagent, and a portion of the colony under study was transferred to the filter paper using wooden sticks. A positive result was indicated by the appearance of a purple colour upon contact of the bacterial cells with the reagent on the paper (15)

2.3 Bacterial Diagnosis with the Vitek 2 Compact Device

The Vitek device is considered one of the best devices for identifying bacterial species quickly and accurately. Developed by the French company Biomeriex, it identifies the type of bacteria by performing 64 tests.

2.4 Antimicrobial Susceptibility Testing

Kirby–Bauer disk diffusion on Mueller-Hinton agar using CLSI (2023) guidelines. Antibiotics tested included:

Concentration M icrograms /tablet	Code	Antibiotics
10	**CRO**	**Ceftriaxone**
30	**NV**	**Novobiocin**
30	**EM**	**Erythromycin**
30	**AMC**	**Amoxicillin/clavulanic acid**
10	**IPM**	**Imipenem**
5	**OFX**	**Ofloxacin**
30	**VA**	**Vancomycin**
25	**NA**	**Nalidixic acid**
10	**CTX**	**Cefotaxime**
5	**CFM**	**Cefixime**

Table 1.

** **MDR was defined as resistance to ≥3 antibiotic classes .**

Results

3.1 Sample Collection , Isolation , and Identification

Between February and March of 2023, fifty samples were taken from pregnant and non-pregnant women at Al-Khansaa Hospital in Mosul City and Al-Batoul Teaching Hospital in Diyala Governorate. These samples comprised 15 (30%) non-pregnant women and 35 (70%) pregnant women who showed clinical signs of bacterial vaginosis (BV). The samples were examined morphologically, microscopically, biochemically, and molecularly.

3.2 Morphological Identification

The attending physician and laboratory technician collected clinical samples and categorized them according to the color, odour, and pH of vaginal discharge using a pH meter. According to the findings, the colour of the vaginal discharge varied from white to greenish-yellow (20%, 35%, and 45%, respectively). The vaginal discharge has a pH between 5.0 and 7.0. Based on their physical traits, the bacterial species responsible for bacterial vaginosis (BV) were first identified (Figure 4-1). Blood agar, chocolate agar, and MacConkey agar were used for direct culture of the samples, which were then incubated for 24 hours at 37°C. Twenty (40%) of the samples had positive bacterial growth, according to the results, and thirty (60%) had mixed growth (fungi and yeast), which led to their exclusion from the research.

The Vitek assay was used to authenticate the morphological and biochemical identities of the samples. While some isolates appeared white or grey on blood agar and chocolate agar, others exhibited a pink colour on MacConkey agar, suggest916ing their capacity or inability to ferment lactose. (Vaginal Infections Atlas [STDs], 2018) (16)

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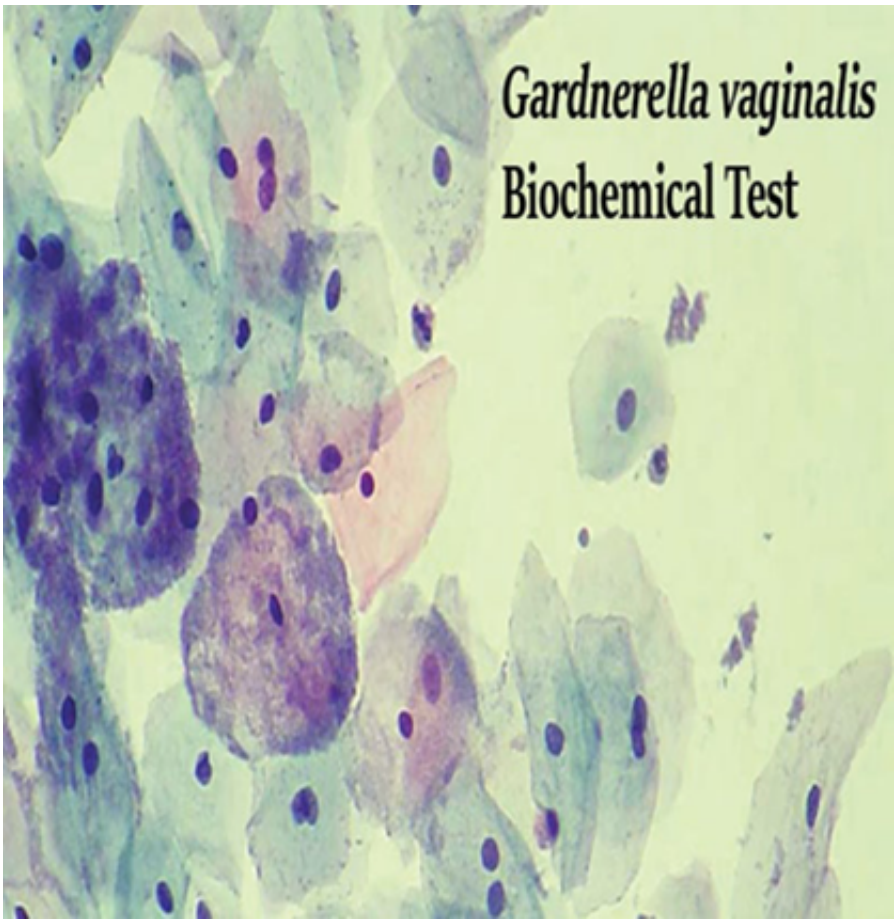

Figure 1. **Figure (3- 1) Clinical samples of vaginal swabs: A) Positive for BV and B) Negative for BV**

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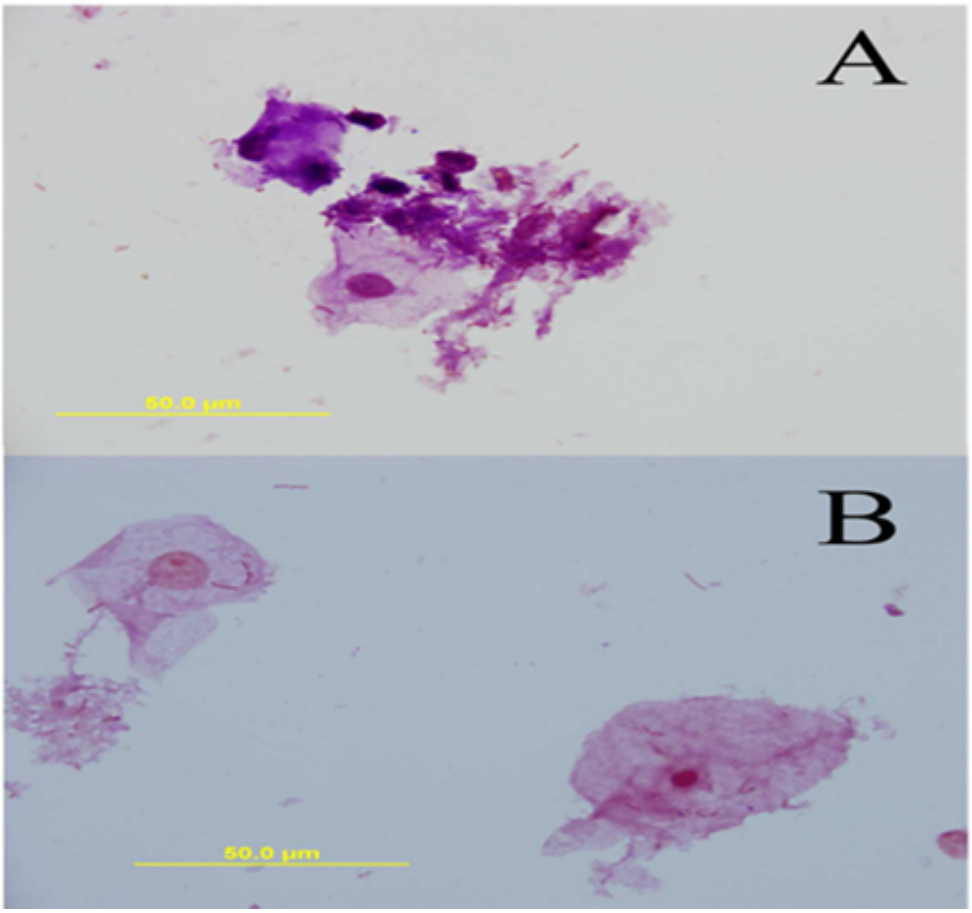

Figure 2. **Figure (3- 1) Clinical samples of vaginal swabs: A) Positive for BV and B) Negative for BV**

3 .3 Microscopic Examination

The bacteria appeared under the light microscope at a magnification of 100x after staining with Gram's stain. Gram-negative bacteria appeared red, and Gram-positive bacteria appeared purple (Figure 4-2). The percentage of Gram-positive bacteria (65%) was higher than the percentage of Gram-negative bacteria (35%), as shown in Table 4-1 and Figure 3-1.

Gram negative	Gram positive	%	Bacterial isolates

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