Original Article
DOI: https://doi.org/10.70749/ijbr.v3i2.614

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Association of Gestational Diabetes Mellitus with Multi Drug Resistant Vaginal Infections among Pregnant Women

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ARTICLE INFO

Keywords

Bacterial Vaginosis, MDR, Gestational Diabetes Mellitus, Gram-positive Bacteria, Gram-negative Bacteria, Candida.

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Declaration

Author's Contributions: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History

Received: 08-12-2024
Revised: 27-01-2025
Accepted: 02-02-2025

ABSTRACT

Background: Vaginal infections caused by various pathogenic microorganisms, along with the emergence of antibiotic resistance, pose significant medical challenges. Gestational diabetes mellitus (GDM) is a critical factor that can delay recovery from infections and lead to complications. **Methodology:** This study was conducted as a cross-sectional analysis involving pregnant women. A total of 120 vaginal swabs were collected from participants and analyzed to identify the microorganisms present and assess their antibiotic susceptibility. The subjects were categorized into two groups based on their diabetic status: those with GDM and those without. Various biochemical tests, culturing methods, and sensitivity patterns were performed on high vaginal swab isolates. **Results:** The analysis revealed that 56% of the women studied were aged between 23 and 37 years, with 67% in their third trimester. Among the microorganisms identified, Candida species accounted for 51%, while gram-positive bacteria comprised 24.3% and gram-negative bacteria 25.19%. Within the gram-positive category, Methicillin-resistant Staphylococcus aureus (MRSA) was the most prevalent at 30.25%, followed by Enterococcus faecalis at 15% and Streptococcus species at 3.11%. For gram-negative bacteria, Klebsiella pneumoniae was found in 44.4% of cases, E. coli in 31.3%, Pseudomonas aeruginosa in 14.1%, Pseudomonas stutzeri in 6.05%, and Acinetobacter baumannii in 3.02%. Susceptibility testing indicated that Staphylococcus aureus was primarily sensitive to Linezolid but resistant to azithromycin, while vancomycin and cotrimoxazole were most effective against MRSA, with cloxacillin showing significant resistance. Vancomycin was also the most effective treatment for E. faecalis, whereas azithromycin exhibited high resistance levels. Among gram-negative bacteria, Gentamicin was most effective against E. coli, while Rofloxacin showed the highest resistance; Imipenem was most effective against P. aeruginosa, with Amikacin showing significant resistance. A significant association was found between multidrug-resistant (MDR) vaginal infections and GDM. **Conclusion:** The study concluded that there is no significant association between MDR vaginal infections and gestational diabetes mellitus among pregnant women.

INTRODUCTION

The vagina is inhabited by a diverse array of beneficial microorganisms, collectively known as the vaginal microbiota. This community includes bacteria, fungi, viruses, and archaea, all of which are essential for maintaining vaginal health and homeostasis while protecting against infections. The presence of glycogen in the vaginal environment exerts selective pressure that influences which microorganisms can thrive (1-3). The composition and stability of the vaginal microbiota can be affected by various factors, including dietary habits, hormonal changes, sexual activity, hygiene practices, antibiotic use, and different life stages such as pregnancy (4, 5).

In a healthy state, the vaginal microbiota typically displays low diversity and is predominantly composed of Lactobacillus species. Approximately 70% of women have dominant species such as Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus jensenii, or Lactobacillus iners in their vaginal microbiota (6). A microbiota dominated by Lactobacillus is considered a marker of vaginal health, correlating with a lower risk of infections like bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and sexually transmitted infections (7). Different Lactobacillus species offer varying degrees of protection; for example, L. crispatus is associated with stable vaginal flora and reduced infection risk, while L. iners is more prevalent in dysbiotic conditions (8).

The vaginal pH typically ranges from 3.8 to 4.5 due to lactic acid production by Lactobacilli, creating an acidic environment that inhibits bacterial overgrowth (9). A pH above 4.5 may indicate various conditions such as hormonal fluctuations during the menstrual cycle or infections like BV and VVC (10). Symptoms associated with these conditions can include purulent discharge, inflammation, and discomfort (11).

Dysbiosis in the vaginal microbiota—characterized by an imbalance in Lactobacilli—can disrupt microbial equilibrium and increase susceptibility to infections. Factors contributing to dysbiosis include antibiotic use, hormonal fluctuations influenced by estrogen levels, sexual behaviors, and douching practices that disturb normal vaginal flora (12). Vaginal infections are marked by inflammation and discomfort due to an overgrowth of pathogenic microorganisms such as E. coli, Klebsiella spp., Staphylococcus aureus, and Candida species (13). Pregnancy can further heighten the risk of these infections due to physiological changes that favor microbial growth (14).

Bacterial vaginosis is a common condition among reproductive-age women characterized by a disruption of normal vaginal flora and an overgrowth of anaerobic bacteria. BV has been linked to adverse pregnancy outcomes such as preterm birth and low birth weight (15). Similarly, vulvovaginal candidiasis, primarily caused by Candida albicans, affects many women throughout their lives and is particularly prevalent during pregnancy (16).

Understanding the complexities of the vaginal microbiota and its impact on female reproductive health is vital for developing effective prevention and treatment strategies for vaginal infections. This study aims to investigate the relationship between dysbiosis and the incidence of bacterial vaginosis and candidiasis during pregnancy to enhance our understanding of these common conditions.

MATERIALS AND METHODS

Study Design and Setting

This is a cross-sectional study that was conducted at 3 different THQ hospitals in Rawalpindi. The research was carried out over four months, from June, 20224 to November 2024, after research board approval. Data collection occurred three times a week.

Selection Criteria

Inclusion Criteria: Pregnant females with active vaginal site infections, with or without gestational diabetes.

Exclusion Criteria: Non-pregnant females with vaginal infections.

Sample Size

A total of 120 patient specimens were analyzed during the study period.

Sampling Techniques

Sample Collection

High vaginal swab samples were collected using a sterile speculum and swab stick under gynecologist supervision. Samples were labeled with patient details and transported to the microbiology lab for analysis.

Culturing of Specimens

Swabs were inoculated on blood, chocolate, and MacConkey agar plates and incubated aerobically for 18-24 hours. Bacterial growth was identified based on colony morphology.

Microscopic Identification

Gram-positive and Gram-negative bacteria were identified through microscopic examination.

Gram Staining

Gram staining was performed using the following steps:

1. Crystal violet stain application.
2. Iodine mordant application.
3. Alcohol decolorization.
4. Safranin counterstaining.

Biochemical Tests

Catalase Test

A small colony was mixed with 3% H_2O_2 on a slide to observe bubble formation, indicating catalase activity.

Coagulase Test

Fresh cultures were emulsified in plasma and incubated to observe clot formation, indicating a positive result.

API 20e

A single colony was suspended in sterile water, filling the API 20e strip compartments, and incubated at 37°C for 18-24 hours.

Antibiotic Susceptibility Test (AST)

Antimicrobial susceptibility was assessed using the Kirby-Bauer Disc Diffusion method on Mueller-Hinton agar. The procedure included preparing the agar plates, inoculating with the test organism, placing antibiotic disks, and incubating at 35°C for 16-24 hours. Zones of inhibition were measured post-incubation.

Germ Tube Assay

Yeast colonies were suspended in fetal bovine serum, incubated, and examined for germ tubes under a microscope.

Screening for Gestational Diabetes

The Oral Glucose Tolerance Test (OGTT) involved fasting participants, measuring baseline blood sugar, administering a glucose solution, and subsequent blood sampling.

Statistical Analysis

Data were analyzed using SPSS version 22.0, with categorical data expressed as frequencies.

RESULTS

A total of 120 participants with vaginal discomfort were included in the study. The exclusion criteria eliminated women with negative pregnancy outcomes. The peak prevalence of vaginal dysbiosis was observed in the 28-37 years' age group (58%), while the lowest prevalence was in the 38-47 years age group (17.4%) (Figure 5).

The prevalence of vaginal infections varied significantly across age groups: 18-27 years (23.01%), 28-37 years (57.1%, peak prevalence), and 38-47 years (17.4%).

The severity of vaginal infections was highest in the third trimester (68.2%), while the first trimester had the lowest prevalence (7.14%). The second trimester showed a prevalence of 24.6% (Figure 6).

Microbial analysis of high vaginal swab (HVS) samples revealed the following: Candida species (52.3%), Gram-positive bacteria (25.3%), and Gram-negative bacteria (26.19%).

Gram staining identified the following Gram-positive bacteria: Staphylococcus aureus (50%), Enterococcus faecalis (16%), and Streptococcus species (3.12%). The predominant Gram-negative bacteria included Klebsiella pneumoniae (45.4%), Escherichia coli (30.3%), and Pseudomonas aeruginosa (15.1%).

Biochemical tests confirmed Staphylococcus aureus as coagulase-positive, Enterococcus faecalis and Streptococcus spp. as catalase-negative, and API 20E results indicated specific biochemical reactions for various bacteria.

Antibiotic susceptibility was assessed according to CLSI guidelines. Key findings include Staphylococcus aureus being sensitive to vancomycin, cloxacillin, and linezolid; Enterococcus faecalis being sensitive to vancomycin and chloramphenicol; Escherichia coli being highly sensitive to amikacin and cefoperazone; and Klebsiella pneumoniae being sensitive to imipenem and amikacin.

Among pregnant women with vaginal infections, 13.4% had gestational diabetes, while 86.5% did not. The occurrence of GDM during different trimesters was 3.9% in the 2nd trimester and 9.5% in the 3rd trimester.

Candida infections had a 10.6% prevalence in GDM cases, with Gram-positive bacteria showing a 25% GDM prevalence and Gram-negative bacteria showing a 6.06% GDM prevalence. These results indicate that vaginal infections are more common in non-GDM patients across all infection types.

Figure 1

Age wise distribution

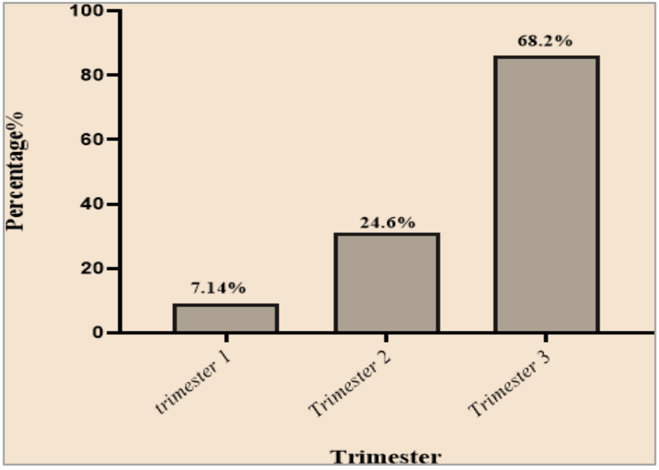


Figure 2

Prevalence of Vaginal Infections Across trimesters

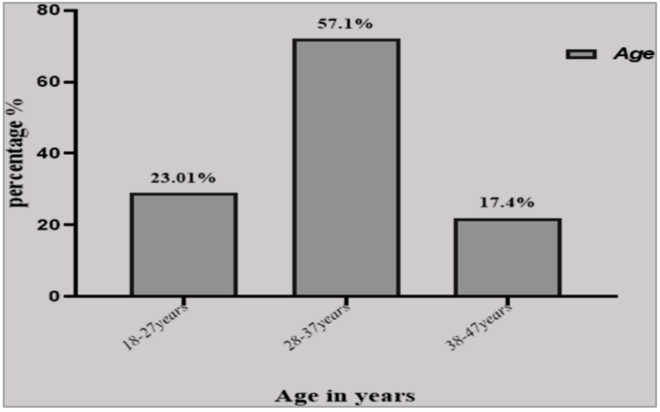


Figure 3

Prevalence of Vaginal Infections among Pregnant Women

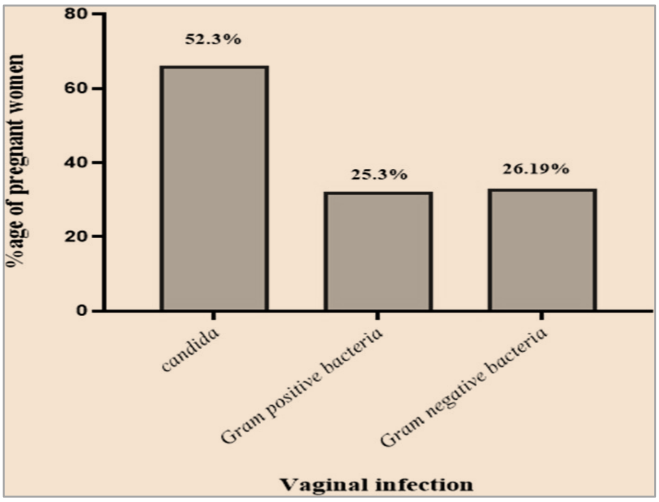


Figure 4
Percentage of Gram-positive bacteria isolated from HVS specimen.

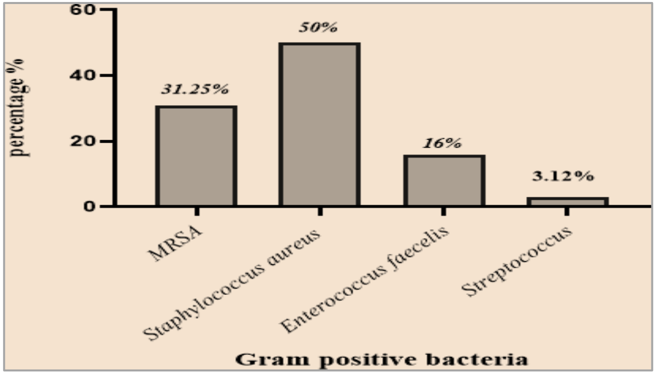


Figure 5
Percentage of Gram-negative bacteria isolated from vaginal infections

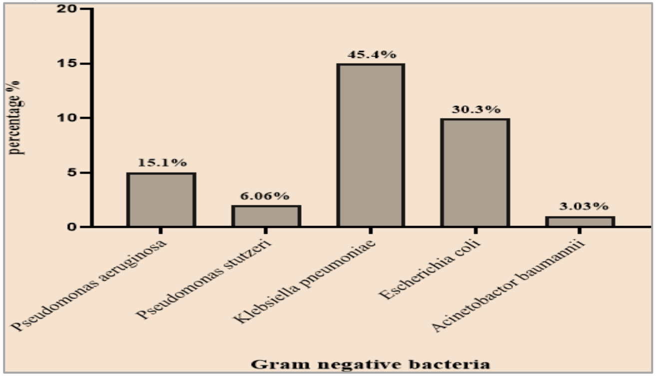


Figure 6
Antibiotic Sensitivity pattern of MRSA

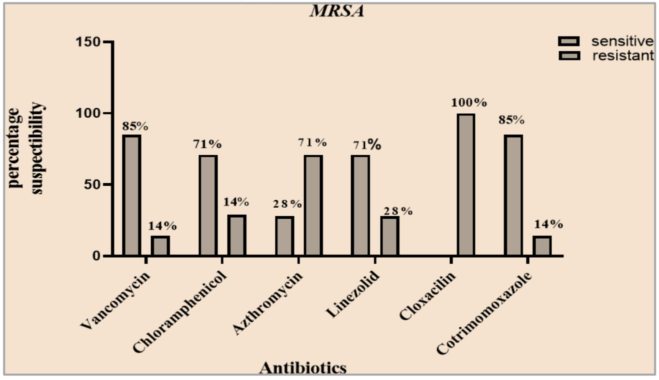


Figure 7
Antibiotic Sensitivity pattern of Enterococcus faecalis

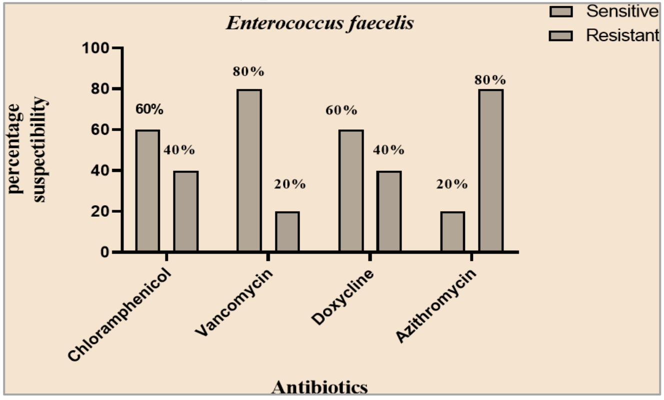


Figure 8
Antibiotic Sensitivity pattern of E,coli

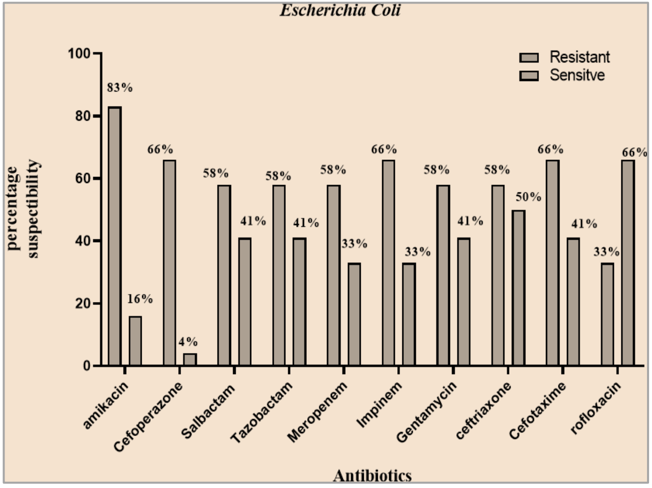


Figure 9
Antibiotic Sensitivity pattern of Pseudomonas aeruginosa

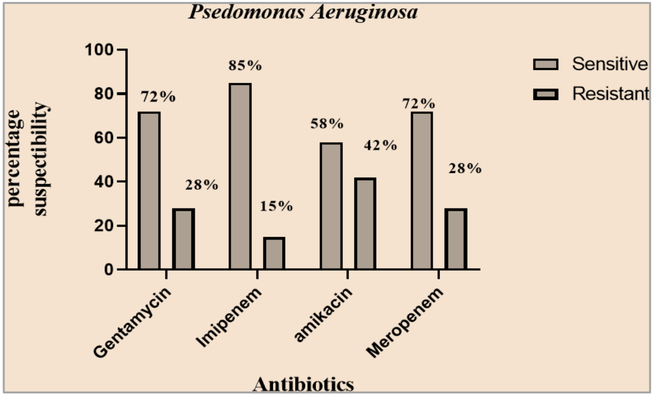
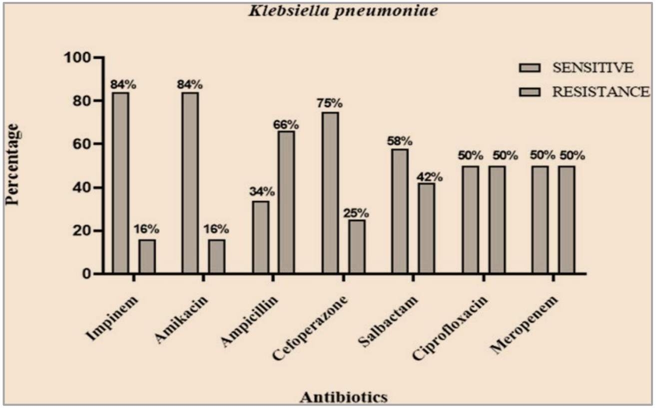


Figure 10
Antibiotic sensitivity pattern of Klebsiella pneumoniae



Association of MDR vaginal infections with GDM

Our study investigated the association between multidrug-resistant (MDR) vaginal infections and Gestational Diabetes Mellitus (GDM) using the χ^2 test. The results showed a significant association, with a p-value of 0.025, indicating that MDR vaginal infections are more prevalent among non-GDM pregnant women. Specifically, among the infected pregnant participants, 10.6% were diagnosed with GDM, while 89.3% were normoglycemic.

Furthermore, bacterial infections were notably more frequent in women without GDM during the second and third trimesters. However, persistent bacterial infections were significantly less common in those with GDM. Additionally, vaginal candidiasis was found to be significantly more prevalent in women without GDM across both trimesters, including cases of persistent infections.

CONCLUSION

This study involving 120 pregnant women with multidrug-resistant (MDR) vaginal infections identified the most prevalent organisms as MRSA (31.25%), Escherichia coli (30%), and Klebsiella pneumonia (45%). Antibiotic susceptibility testing indicated significant resistance patterns among these organisms. Notably, the prevalence of Gestational Diabetes Mellitus (GDM) in women with yeast infections was 10.6%, while 25% of women with GDM had vaginal infections compared to 75% of those without GDM. Overall, the findings suggest that there is no significant association between MDR vaginal infections and gestational diabetes mellitus.

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