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Article

Impact of IUCD Use on Vaginal Microbiota and Antibiotic Resistance: A Comparative Study Among Iraqi Women

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Abstract

Background: Bacterial vaginosis (BV) is a prevalent vaginal infection affecting millions of women worldwide, often associated with an imbalance in vaginal microbiota. The use of intrauterine contraceptive devices (IUCDs) has been linked to alterations in vaginal flora, increasing the risk of infections. However, data on the bacterial composition and antibiotic susceptibility patterns in IUCD users compared to non-users remain limited, particularly in Iraqi women. ***Objectives:*** This study aims to assess the prevalence of vaginal bacterial colonization in IUCD users versus non-IUCD users and determine the antibiotic resistance patterns among Gram-negative bacterial isolates. Additionally, the study evaluates vaginal pH variation ***Methodology:*** The study was conducted in a private gynecology clinic in Dhi Qar Governorate, Iraq, from November 2022 to June 2024. A total of 170 women (99 IUCD users and 71 non-IUCD users) were enrolled. High vaginal swabs (HVS) were collected and analyzed using the VITEK® 2 compact system (bioMérieux, France) to identify bacterial species and assess antimicrobial susceptibility. Vaginal pH was measured using litmus paper. ***Results:*** The vaginal pH range (3-6) showed no significant difference ($P > 0.05$) between IUCD and non-IUCD users. However, IUCD users had a significantly higher bacterial load. Escherichia coli was the most common Gram-negative isolate in both groups. ***Conclusion:*** This study underscores a higher prevalence of bacterial colonization and antimicrobial resistance in IUCD users, particularly against β-lactams and fluoroquinolones. Culture-based antibiotic therapy is recommended to prevent treatment failure in vaginal infections. Further research is needed to evaluate the long-term effects of IUCD use on bacterial biofilm formation and antibiotic resistance evolution.

Keywords: IUCD, Bacterial vaginosis, Vaginal microbiota, Antimicrobial resistance, Multidrug-resistant bacteria (MDR), Biofilm formation, Gram-negative bacteria, Antibiotic susceptibility testing, Bacterial infections, Vaginal pH.

1. Introduction

The female genital tract hosts a diverse microbial ecosystem, with bacterial vaginosis (BV) commonly associated with intrauterine device (IUD) use [1]. However, limited research explores its correlation with oral contraceptives and condoms [2]. BV is an opportunistic infection influenced by microbial virulence and host factors such as immunity, age, diet, and stress While some bacteria are harmless, pathogens can invade and cause illness through toxicity and infiltration [3-5].

Antibiotic use has shaped bacterial evolution, reduced pathogenic populations but also increased resistance, leading to significant public health concerns [6]. Globally, antibiotic-resistant infections account for 44,000 deaths and 2.6 million cases annually [7, 8]. Effective BV prevention and management require early detection and timely treatment to mitigate its impact [9]. Contraception is widely used to prevent pregnancy, with 99% of sexually active women utilizing it [10]. Various methods, including devices, drugs, and medical procedures, are available, with effectiveness, safety, and accessibility being key considerations [11]. Long-acting reversible contraceptives (LARCs), such as subdermal implants and intrauterine devices (IUDs), have a failure rate of less than 1% [12].

Contraceptive injections contain progesterone to inhibit fertilization, while hormonal options include pills, patches, and rings [13, 14].

Contraceptive efficacy depends on both the method's inherent effectiveness and potential user errors. Understanding these factors helps assess the reliability of different contraceptive options [15].

Some studies suggest that IUD users may have an increased risk of BV, particularly with levonorgestrel-releasing intrauterine systems (IUS) or copper IUDs [16]. Additionally, IUDs can raise the risk of pelvic inflammatory disease (PID) and its complications, like infertility and ectopic pregnancy [17]. The presence of biofilms, where bacteria and host cells are embedded, on IUDs may contribute to increased infections [18].

Research is exploring the relationship between vaginal pH and contraceptive methods18, as vaginal pH can influence the vaginal microbiome and overall vaginal health [19]. Hormonal contraceptives, such as combined oral contraceptives and progestin-only methods (e.g., injectables and implants), may increase vaginal pH by reducing Lactobacillus species, potentially raising the risk of infections like bacterial vaginosis (BV) [20, 21]. Non-hormonal methods, like condoms and diaphragms, can also affect pH when used with spermicides, which may disrupt the natural vaginal flora and elevate pH, increasing infection susceptibility [22, 23].

There is limited information on the antimicrobial susceptibility of microorganisms found in women using contraceptives [24]. Studies have shown that bacteria from these women often exhibit high resistance to antimicrobial drugs, likely due to previous antibiotic exposure and the bacteria's ability to develop resistance [25, 26]. The use of contraceptives can contribute to the development of resistance26. Understanding the susceptibility profiles of bacteria in contraceptive users is essential for preventing treatment failures and guiding appropriate empirical therapy for infections [27]. Early identification of resistant bacteria is crucial in managing potential infections in these women [28].

Multidrug resistance (MDR) in bacteria is facilitated by various mechanisms, including changes in the bacterial membrane that either reduce drug uptake or increase drug efflux, as well as drug inactivation or modification [29]. Gram-negative bacteria, in particular, are a significant concern in MDR, as they reduce the efficacy of many antibiotics [30]. The main mechanisms of MDR in these bacteria involve efflux pumps, which are proteins that expel antibiotics from the bacteria [31]. These pumps include the ABC transporters, SMR, MATE, MFS, and RND families, with the RND family being common in E. coli and other gram-negative bacteria [32, 33].

2. Materials and Methods

The study was conducted in Dhi Qar Governorate, with samples collected from 170 women (86 IUCD users and 71 non-IUCD users) at a private gynecology clinic between November 2022 and June 2024. The participants, aged 18-45, were healthy, had not used antibiotics or vaginal washes in the past two weeks, and were not using any vaginal wash. High vaginal swabs were taken from the posterior fornix of the vagina using sterile swabs immersed in distilled water. After sampling, the swabs were placed on the vaginal wall for 20 seconds, then transferred to a sterile container and sent to the lab [34-38]. Litmus paper was used to measure vaginal pH, and a screening questionnaire ensured the absence of antibiotic or vaginal wash use within the previous two weeks.

2.1 The Identification of Bacteria and the Evaluation of Antibiotic Susceptibility

The automated VITEK® 2 compact system (bioMérieux, France) was used to identify bacteria and evaluate their antibiotic susceptibility using AST-N222 and AST-P580 cards. Isolates of clinical significance were subjected to a subculturing process to ensure purity before implementing the VITEK® system. These isolates were inoculated onto nutrient and blood agar and incubated at 37°C overnight under aerobic/anaerobic conditions. Colonial morphology and Gram staining classified bacteria. After overnight incubation, the purified bacterial colonies were utilized to get a standard saline inoculum for the appropriate VITEK® identification (ID) card. The identification of bacteria was conducted using the following ID cards: Gram-negative (GN reference 21 341). The MICs and the ASTs were determined using specific sensitivity cards, also called AST cards. According to the CLSI's criteria, the susceptibility tests were interpreted using the VITEK® 2 and advanced expert systems (AES). the AST cards was for Gram-negative bacteria (AST-N222). All processing procedures were conducted following the manufacturer's instructions. A VITEK® 2 Compact was used to log and load ID and AST cards. Version 06.01 of the VITEK® 2 Systems software automatically reports and prints the data obtained from the VITEK® 2 Compact system [39].

Gram-negative bacteria were tested against AST-N222, including: Ampicillin (AM), Piperacillin/Tazobactam (TZP), Cefazolin (CZ), Cefoxitin (FOX), Ceftazidime (CAZ), Ceftriaxone (CRO), Cefepime (FEP), Ertapenem (ETP), Imipenem (IPM), Amikacin (AN), Gentamicin (GM), Ciprofloxacin (CIP), Levofloxacin (LEV), Tigecycline (TGC), Nitrofurantoin (FT), and Trimethoprim/Sulfamethoxazole (SXT).

2.2 Data Analysis

Utilizing Microsoft Excel (2021), the data was submitted and analyzed. The similarity index (Dice coefficient) and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Clustering method.

2.3 Ethical Consideration

The AL-Nasiriya Health Department granted ethical approval for this study, which the Research and Development Department subsequently approved. Vaginal samples were collected under the supervision of a gynecologist after patient consent was collected.

3. Results and Discussion

3.1 Participants in the Study

From November 2022 to June 2024, the Laboratory of Microbiology at the Al-Nasiriyah Teaching Hospital examined high vaginal swabs (HVS) from 170 women, including 99 women with IUCD and 71 non-IUCD Women.

3.2 The Vaginal pH Values

The vaginal pH range (3-6) was the same for IUCD- and non-IUCD-using women. There was no significant difference ($P < 0.05$) between the both groups Fig. 1.

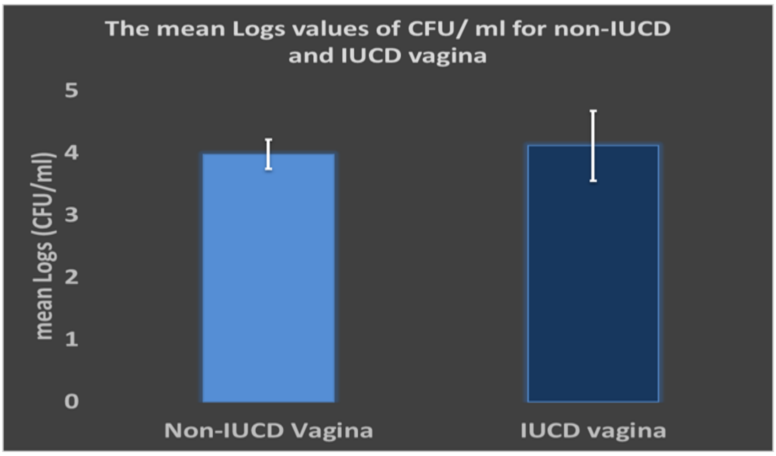

Figure 1. the Mean Logs Values of CFU/ml non-IUCD and IUCD Vagina.

3.3 Bacterial abundance

The mean value cultivable bacteria count in non-IUCD-using women was 3.97 logs, while in IUCD-using women, it was 4.11 logs, showing a statistically significant difference ($P<0.05$) Fig. 2.

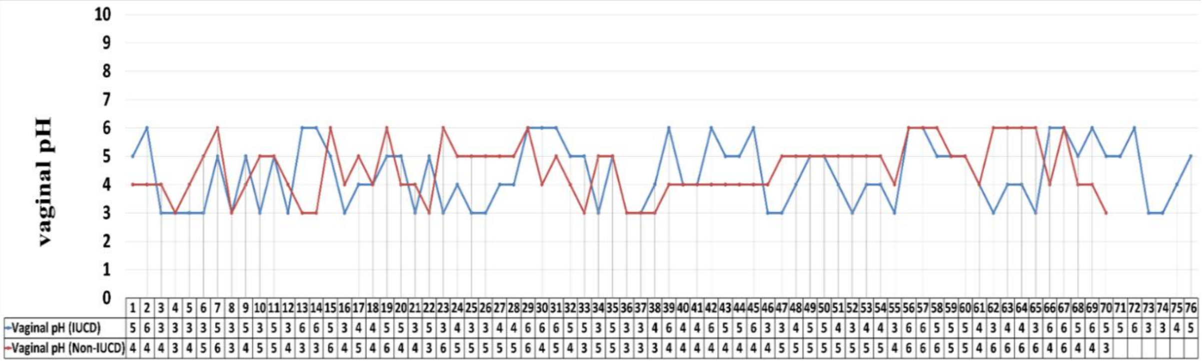

Figure 2: Total cultivable bacterial communities were distributed in the non-IUCD-using and IUCD-using women, expressed in mean Logs (CFU/ml). Whisper: mean ± standard deviation. P-value (<0,05)

3.4 Bacterial diversity

Our results indicate that among 99 vaginal samples from IUCD-using women, 13 samples (15.7% of IUCD-using women) were unidentified bacteria. In a study involving 71 vaginal samples from non-IUCD-using women, 11 vaginal samples (14.5%) were classified as undetermined bacteria. There were unidentified samples was 24, constituting 15.09% of the total collected samples.

The VITEK® 2 compact system detected 31 Gram-negative bacteria in 86 vaginal samples from women using IUCDs. In comparison, 60 samples from non-IUCD users contained 28 Gram-negative bacteria, as shown in Tables 1 and 2.

Ten Gram-negative species were identified in IUCD-using women's samples, and E. coli was the most prevalent (8 (11.4%), as demonstrated in Table 1.

In non-IUCD-using women's samples, 9 Gram-negative organisms were detected. However, the results showed the same most prevalent species found in the samples of IUCD-using women, but in different percentages: Enterococcus faecalis (8, 12.3%) and E. coli (10, 15.4%), as illustrated in Table 2. In contrast, Fig. 3 shows thirteen species in both IUCD-using and non-IUCD-using women.

Table 1. GN bacteria isolates in high vaginal samples of IUCD-using women frequency distribution and percentages (N=80)

IUCD-using women (N=86)	
Genus or Species (Gm -ve)	Frequency (%)
E. coli	8 (11.4%)
Klebsiella pneumoniae	4 (5.7%)
Enterobacter cloacae	5 (7.1%)
Pantoea sp.	2 (2.9%)
Proteus mirabilis	2 (2.9%)

Table 2. GN bacteria isolate in high vaginal samples of non-IUCD-using women frequency distribution and percentages (N=60)

Non-IUCD-using women (N=60)	
Genus or Species (Gm -ve)	Frequency (%)
E. coli	10 (15.4%)
Enterobacter cloacae	4 (6.2%)
Klebsiella pneumoniae	3 (4.6%)
Pantoea sp.	2 (3.1%)
Acinetobacter haemolyticus	2 (3.1%)

Antimicrobial susceptibility profile of Gram-Negative bacteria isolated from a non-IUCD-using woman

UPGMA cluster analysis revealed five clusters show 50% similarity among the isolated Gram-negative bacteria from women not using IUCDs. All C1 and C2 cluster isolates showed intermediate susceptibility, with few isolates present. All bacterial isolates within the C3 cluster showed sensitivity. All bacterial isolates in the C4 and C5 showed resistance (Fig. 3A).

The susceptibility of Gram-negative isolates to 16 different antibacterial drugs was tested in Fig. 3B.

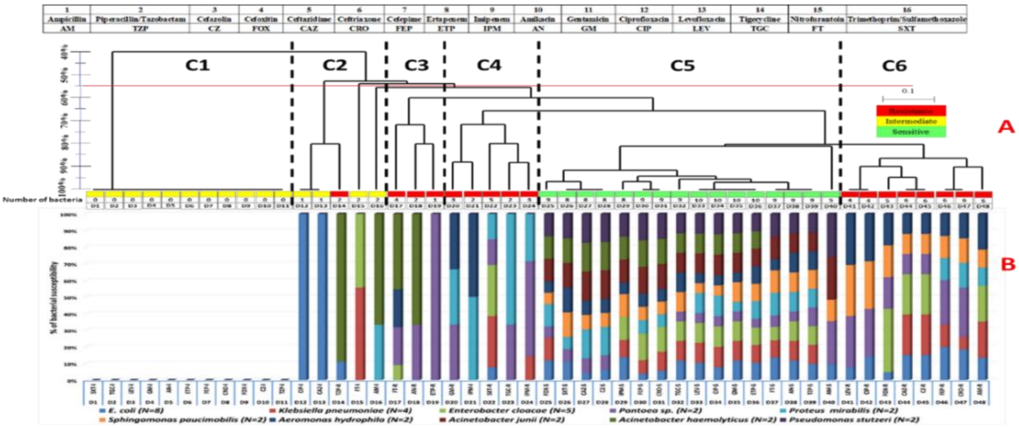

Figure 3. A) UPGM cauterization of gram-negative bacteria isolated from a non-IUCD-using woman defined to the genus or species level and compared to percentage susceptibility to 16 antibacterial drugs. Clusters were defined at 50% similarity. B) non-IUCD-using women bacterial diversity presented in percentage susceptibility (%) with the number of bacteria.

Tests were conducted on 28 Gram-negative isolates, and the following antibiotics were found to be completely resistant (100%): Fox was (11[39.3%]) followed by ETP, CRO and CAZ (7[25%]), IPM, FEP, CZ, and AM (5[17.9%]), FT, GM, AN, TGC, SXT, and TZP (3[10.7%]), LEV, and CIP (1[3.6%])

The Gram-negative isolates showed a 100% sensitivity to AN (25[89.3%]), TGC (23[82.1%]), FT (18[64.3%]), GM, ETP, and TZP (15[53.6%]), CRO, CAZ, LEV, CIP, FEP, and CZ (11[39.3%]), SXT and IPM (9[32.1%]), FOX (7[25%]), and AM (4[14.3%]).

Our study reveals that E. coli was the most prevalent Gram-negative bacteria in non-IUCD women. It showed significant sensitivity (100%) to TGC, FT, AN, and ETP.

The most resistant bacteria observed in our results were Sphingomonas spp., which showed 100% resistance to 16 antibiotics, followed by Pantoea spp., which showed resistance to 10 antibiotics. The bacterial isolates demonstrated multidrug resistance to at least one antibiotic, as noted in Enterobacter cloacae.

Antimicrobial susceptibility profile of Gram-Negative bacteria isolated from an IUCD-using woman

Six UPGMA clusters were identified with 55% similarity regarding isolated Gram-negative bacteria from women utilizing IUCDs. Type bacterial species were significantly associated with antibiotic susceptibility, as demonstrated by the diversity of Gram-negative bacteria structures. The C1 and C2 Clusters consist of a few isolates that showed intermediate. All C3, C4, and C6 cluster isolates exhibited resistance and showed bacterial counts ranging from 2 to 6, while all C5 cluster isolates showed sensitivity and presented bacterial counts between 5 and 10 Fig. 4A. Sixteen antibacterial drugs were used to evaluate the susceptibility of Gram-negative isolates Fig. 4 B.

31 Gram-negative isolates were found to have total resistance (100%) against AM (11 [35.5%]), followed by SXT, CAZ, and CZ (9 [29%]), FOX (5[16.1%]), TZP, FT, AN, TGC, IPM, and FEP (2[6.5%]).

The Gram-negative isolates showed a sensitivity of 100% to ETP (29[93.5%]), TGC and AN (27 [87%]), GM (25[80.6%]), IPM and TZP (21[67.7%]), LEV, and CIP (17[54.8%]), FT (16 [51.6%]), FOX (12[38.7%]), FEP, and CRO (11[35.5%]), SXT (10[32.3%]), CAZ and CZ (8 [25.8%]), and AM (6[19.4%]).

Our study reveals that E. Coli was the most prevalent Gram-negative bacteria in IUCD-using women. It showed significant sensitivity (100%) to IMP, TGC, GM, ETP, FT, and AN.

Enterobacter cloacae has shown resistance to 5 antibiotics, while Klebsiella pneumoniae has resisted 4 antibiotics. Conversely, Proteus mirabilis and Aeromonas hydrophilia have shown resistance to one antibiotic.

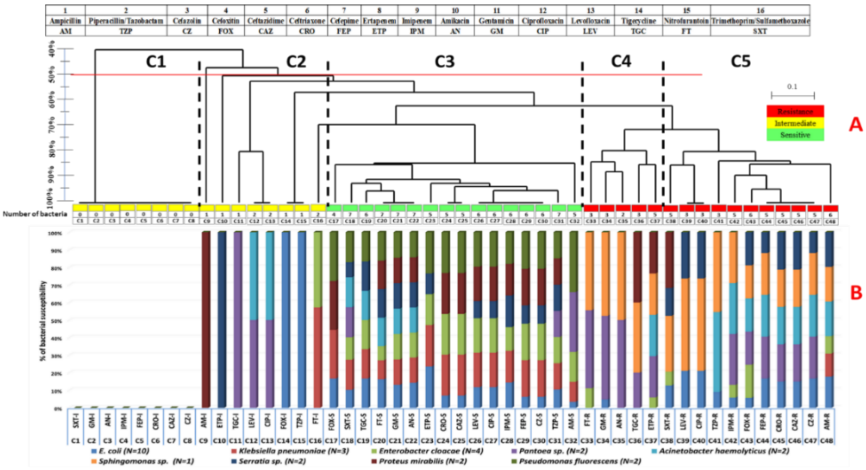

Figure 4. A) UPGM cauterization of gram-negative bacteria isolated from a non-IUCD-using woman defined to the genus or species level and compared to percentage susceptibility to 16 antibacterial drugs. Clusters were defined at 55% similarity. B) non-IUCD-using women bacterial diversity presented in percentage susceptibility (%) with the number of bacteria.

4. Discussion

An outbreak of bacterial vaginosis (BV) increases the likelihood of developing an acute upper vaginal tract infection [38,39]. Two key factors associated with BV acquisition are intermediate vaginal flora at the start of contraceptive use and irregular vaginal bleeding within the first six months of contraceptive use [40].

In this study, vaginal pH was assessed as a diagnostic indicator for vaginal infections [41]. pH can be measured using electrodes or indicator strips, with strips being a cost-effective and highly accurate method [42]. Optimal vaginal pH levels range between 3.8 and 4.5, maintained by acid-base transporters such as Na+/H+ exchangers, HCO3– cotransporters, proton pumps, and Na+ transporters [43].

The study found that both IUCD users and non-users had a vaginal pH range of 3-6, with no significant difference ($P < 0.05$). This aligns with Peebles et al. in 2021 [44], who reported that IUCDs do not alter vaginal pH levels. This suggests that IUCDs do not disrupt the natural acidic environment maintained by Lactobacillus species, which supports vaginal health. The findings may also indicate a lower prevalence of BV among IUCD users, potentially leading to a lower mean vaginal ph. The study also found Escherichia coli to be the most frequently isolated Gram-negative bacteria in both IUCD and non-IUCD users (Tables 1 and 2). However, Odaranle et al. in 2020 [45] reported a lower prevalence of E. coli in high vaginal swabs from women using various contraceptive methods. The high detection rate of E. coli in this study is attributed to anatomical factors, specifically the proximity of the urethra to the anus, facilitating bacterial transfer.

Gram-negative bacteria from both non-IUCD and IUCD users showed complete resistance (100%) to Cefoxitin (Fox) and Ampicillin (AM) at rates of 39.3% and 35.5%, respectively. This aligns with studies suggesting that physiological and environmental factors influence bacterial resistance [46]. 60 IUCDs may alter the vaginal microbiome, promoting the selection of resistant strains due to biofilm formation and chronic inflammation. Additionally, β-lactamase-producing Gram-negative bacteria are prevalent in community settings, indicating that non-clinical antibiotic use may drive resistance in non-IUCD users. Some studies also suggest that copper ions released by IUCDs may reduce antibiotic-resistant bacterial strains [59].

The study found that all tested bacterial species were 100% sensitive to multiple antibiotic classes. The most effective antibiotics against Gram-negative bacteria from both non-IUCD and IUCD users included Amikacin (AN), Tigecycline (TGC), Nitrofurantoin (FT), Gentamicin (GM), Ertapenem (ETP), and others. These findings align with previous studies showing that IUCD use does not significantly impact bacterial susceptibility to antibiotics. Amikacin, Tigecycline, and Nitrofurantoin exhibited 100% sensitivity in both groups, suggesting that IUCDs do not inherently reduce antibiotic effectiveness [47, 48].

The study found that Escherichia coli was the most prevalent Gram-negative bacterium in both non-IUCD and IUCD-user women. It exhibited 100% sensitivity to Tigecycline (TGC), Nitrofurantoin (FT), Amikacin (AN), Imipenem (IPM), Gentamicin (GM), and Ertapenem (ETP). This contrasts with Jain et al. in 2021, who reported a high incidence of multidrug-resistant (MDR) E. coli strains, particularly those resistant to carbapenems and aminoglycosides [49]. The variation may be due to demographic differences, regional antibiotic resistance trends, or selective pressure from widespread antibiotic use in certain areas.

The study found that Sphingomonas spp. exhibited 100% resistance to 16 antibiotic drugs in non-IUCD users. This contrasts with Lv et al. in 2023, who suggested that Sphingomonas resistance is more linked to environmental exposure than contraceptive use [50]. Their research on Sphingomonas in agricultural pests indicated that resistance mechanisms are primarily driven by horizontal gene transfer and symbiotic interactions rather than host-specific factors like contraceptive methods. The discrepancy may stem from differences in sample sources, as this study focused on human clinical samples.

His studies found that Enterobacter cloacae and Klebsiella pneumoniae exhibited 100% resistance to five antibiotics in IUCD users. Possible contributing factors include biofilm formation due to prolonged IUCD use, which shields bacteria from antibiotics and immune responses. Additionally, frequent prophylactic antibiotic use in IUCD users may promote resistant strains, while the lack of routine surveillance and antibiotic stewardship in the region could further drive the spread of multidrug-resistant (MDR) pathogens [51, 52].

5. Conclusions

Escherichia coli were the most common bacteria in both IUCD and non-IUCD users. Gram-negative bacteria were resistant to Cefoxitin and Ampicillin, while Amikacin, Tigecycline, and other antibiotics were effective against them in both groups.

Conflicts of Interest

The authors declare no conflict of interest.

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