

# Prevalence of aerobic bacterial vaginosis among Intrauterine Contraceptive Device users women in Hilla city

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## Abstract:

Intra uterine device (IUD) is the most popular method of contraception among Iraqi women. It is one of the very effective contraceptive methods with very small failure rate. Infection has been the main problem that faces women using IUD all over the world. The aim of this study is to explore the Prevalence of bacterial vaginosis among Intrauterine Device users women in Hilla . A study sample included fifty vaginal swabs taken from women IUD users suffering from vaginosis, who admitted to Babylon hospital for maternity and pediatrics in Babylon province, Iraq during the period from November 2012 to April 2013. These women were randomly selected, interviewed, then cervical swabs were collected and cultured for aerobic bacteria. The results showed that the total number of positive cultures was 32(64%) . The positive cultures predominant among women age between 20-40 years . The first group of isolated organisms was the *Escherichia coli* (16%), followed by *Staphylococcus aureus* and *Klebsiella pneumoniae* (12%) , then *Bacillus subtilis.*, *Proteus mirabilis.*, *Actinomyces israelii.* is 10% , 8% , 6% respectively. The percent of pathogenic swabs decrease with increasing duration of use. The most frequently reported symptom was the presence of abnormal vaginal discharge . When examined for the effects of 5 different antibiotic on bacterial isolated present a variable degree of sensitivity by bacterial isolates .It ranged between 100% to Impenem & Ertapenem and 0% to Penicillin .

**Keywords :** Intrauterine Contraceptive Device, Bacterial Vaginosis, Infection

## الخلاصة:

يعتبر اللولب من اكثر الوسائل شيوعا بين النساء العراقيات ,حيث يعتبر من اكثر الوسائل فاعلية بسبب انخفاض نسبة الفشل في استخدامه , وتعتبر التهابات الجهاز التناسلي من أهم المشاكل التي تواجه السيدات اللواتي يستخدمن اللولب في جميع انحاء العالم ,تهدف هذه الدراسة الى معرفة الانواع المسببة لالتهاب المهبل بين السيدات اللواتي يستخدمن اللولب في مدينة الحلة , تم اختيار العينة الدراسية المكونة من 50 سيدة يستخدمن اللولب والمراجعات مستشفى الولادة والاطفال في محافظة بابل وقد تم اجراء مقابلة شخصية مع كل سيدة للفترة ما بين 2012 و 2013 تم اختيار النساء بصورة عشوائية ومن ثم اخذ عينة من عنق الرحم للزرعة والكشف عن البكتريا الهوائية ببنت النتائج ان النسبة المؤية للعينات موجبة الزرع هي 32 (64%) والنسبة الاكبر كانت في المرحلة العمرية ما بين 20-40 سنة وكانت بكتريا *Escherichia coli* هي الاكثر شيوعا وبنسبة 16% ثم تليها بكتريا *Staphylococcus aureus* بنسبة 12% ثم *Bacillus subtilis* , *Proteus mirabilis* , *Actinomyces israelii* بنسبة 10% , 8% , 6% على التوالي وقد وجد ان نسبة الاصابة تقل مع زيادة فترة استعمال اللولب ,كانت الاعراض المرضية الاكثر شيوعا هي الافرازات المهبلية , تم دراسة تأثير خمسة مضادات حيوية على الاجناس البكتيرية وجد ان هناك درجات متفاوتة من الحساسية للمضادات تراوحت ما بين 100% للمضادين *Impenem & Ertapenem* و 0% لمضاد *Penicillin* .

الكلمات المفتاحية : اللولب , البكتريا المسببة لالتهاب المهبل , اصابة

## Introduction:

Intrauterine devices (IUDs) are highly effective, long-term methods of contraception; and it is also very cost-effective. It is one of the most popular methods of contraception being used by more than 80 million women worldwide, and its effectiveness rivals that of tubal sterilization (Ferraz do *et al.* , 2003 ).

The first modern IUD was introduced by Lippes in 1961. After that, Tatum and Zipper developed the first medicated devices such as Copper 7 and Copper T. Through these devices the copper is dissolved in the uterus. The antifertility effect of copper is the result of the dissolution of copper into uterine secretions (Koumans & Kendrick, 2001).

There are two kinds of IUDs. Both kinds of IUDs work well. One IUD (hormonal) works for up to 5 years. After a few months, women have lighter and fewer periods than they did before, with less cramping. Periods may even stop. The other IUD (copper) can be used for up to 10 years. Women who use this IUD may have heavier bleeding and cramping during their periods, but no side effects from hormones (Neuteboom *et al.*,2003).

However, there are concerns about increased risk of pelvic inflammatory disease (PID) and subsequent complications, such as infertility and ectopic pregnancy, among the IUD users. Some researchers have speculated that the presence of an IUD in the uterus may increase host susceptibility to infection, thus increasing the incidence of PID infections (Yen *et al.* ,2003).

Micro-organisms originating from the normal cervico-vaginal flora then colonize these devices and form biofilms consisting of layers of host cells and bacteria/fungi embedded within a matrix material . The main component of the biofilm produced by the bacteria and/or fungi is an exopolysaccharide layer, which is the pivotal factor responsible for the behaviour of biomaterial centred infection. The biofilm bacteria are usually resistant to attack by antimicrobial agents and host phagocytes. This is one reason why infections caused by these micro-organisms are hard to treat without removal of the devices (Pál *et al.*, 2005).

Marrie and Costerton (1983) used scanning and transmission electron microscopy to study of the surfaces of IUDs. Their transmission electron microscopic study showed highly organized and often densely packed micro-colonies of bacteria, a reflection of the possibility that the majority of these bacteria had been present on these surfaces for a long time. The clinical diagnostic criteria for BV are: vaginal pH greater than 4.5, positive amine test, homogenous gray vaginal discharge (Goffinet *et al.* ,2003).

According to the literature data, women with BV have an increased relative risk of post partum endometritis, post cesarean wound infection, premature rupture of membranes, premature labor, intra-amniotic infection and pelvic inflammatory disease (PID) (Demirezen, 2003).

Reproductive tract infections (RTI) are common among women all over the world. They include three types; the " sexually transmitted disease" , the endogenous infection caused by over-growth of organisms present in the genital tract such as bacterial vaginosis, and finally infections associated with medical procedures that manipulate the genital tract as unsafe abortion, pelvic examination, and IUD insertion (Mumtoz *et al.*,2008) .

Bacterial vaginosis (BV) is the most common vaginal infection among reproductive age women(Joesoef *et al.* ,2001). Use of an IUD has been shown to be associated with BV in many studies. It has been proposed that the tail of the IUD might favor vaginal growth of bacteria associated with BV. In addition, It has also been suggested that the presence of BV during the IUD insertion could also lead to an increased risk of complications in the first 3 months of use including PID(Ness *et al.*,2002) .

This study was aimed to determine the prevalence of Bacterial vaginosis among IUD user.

## **Materials & Methods**

### **Specimen Collection:**

Fifty vaginal swabs taken from women IUD users suffering from vaginitis, who admitted to Babylon hospital for maternity and pediatrics in Babylon province, Iraq during the period from November 2012 to April 2013. Swabs for culture were placed

in tubes containing normal saline to maintain the swabs moist until being taken to the laboratory.

**Culture condition:**

Each specimen was immediately inoculated on blood agar, nutrients agar, MacConkey's agar . All plates were incubated aerobically at 37 °C for 24-48 hours.

Gram stain of smears of each specimen were done after incubation on plates.

Morphologies of these isolates were studied .

**Biochemical test :**

The methods used in the identification and characterization of isolated bacteria included motility test and biochemical tests according to (Cheesbrough, 2004). The isolates were identified by Bergey's Manual for Determinative Bacteriology (Buchanan & Gibbons, 1974)

**Antimicrobial Susceptibility Testing :**

The susceptibility of bacterial isolated to antimicrobial agents was determined using disk diffusion method and interpreted according to National Committee for Clinical Laboratory Standerds (CLSI,2010). The following antimicrobial agents were obtained from (Oxoid, U.K.) as standard reference disks as known potency for laboratory use: Impenem (IMP) 10 µg, Ertapenem (ETM) 10 µg, Gentamycin (GN) 10 µg, Penicillin (P) 30 µg, and Ciprofloxacin (CIP) 5 µg.

All these tests were performed on plates of Muller-Hinton agar (Oxoid, UK). A 0.5 MacFarland suspension (provided by Biomérieux, France) of tested bacterial isolates was applied to the plates, which were dried in an incubator at 37 °C for 15 minutes. Antimicrobial disks were placed on the agar with sterile forceps. The agar plates were incubated inverted at 37 °C for 18 hours. Results were recorded by measuring the inhibition zone (in millimeters) and interpreted according to (CLSI,2010).

**Result and discussion:**

The results showed that the total number of positive cultures was 32(64%), were IUD users . The positive cultures predominant among women age between 20-40 years Table (1).

**Table (1) relationship between infection and age**

Age(years)	Positive culture No.(%)	Negative culture No. (%)	Total No.(%)
<20	6(12)	2(4)	8(16)
20-40	16(32)	10(20)	26(52)
>40	10(20)	6(12)	16(32)
Total	32(64)	18(36)	50(100)

These results can be explained by several previous studies in the past that explained the role of IUD in changing normal vaginal flora. One study related this to the tail and string of the IUD by its effect on transmission of the pathogenic microorganisms and so the role of ascending infection (Omoregie *et al* .,2010) . A second study related the infection to mechanism of action ; considering that IUD produces an inflammatory reaction which thought to interfere with fertilization , at the same time, IUD was considered as any other device or catheter and so has its role in developing infections associated with foreign body ( Christopher *et al* .,2011).

In 1998, Guerreiro and colleagues found a significantly higher prevalence of infection in IUD users in comparison with users of other contraceptive methods, based on the higher prevalence of bacterial vaginosis (BV) in this population.

Moreover, Ferraz do Lago and coworkers (2003) found that the prevalence of cervico-vaginal infections was 29.1% and that BV was frequently found (19.7%) among IUD users 6 months after insertion. The incidence of BV among IUD users

can be even higher (47.2%), as noted by Joesoef et al. (2001). Indeed, IUDs are considered to cause PID by pushing vaginal and cervical bacteria into the uterus along the tail of the device (Ferraz do Lago *et al.*, 2003).

Table (2) shows The first group of isolated organisms was the pathogenic bacteria *E.coli* (16%), followed by *S.aureus* and *K.pneumonia* (12%) , then *B.subtilis*, *P. mirabilis* , *A.israelii* is 10% , 8% , 6% respectively from all positive results.

**Table(2) Number and percentage of bacterial species isolated from Intrauterine Device users women with vaginosis.**

Bacterial species	Single isolated NO.(%)	Mixed isolated NO.(%)	Total isolated NO.(%)
<i>S.aureus</i>	2(4)	4(8)	6(12)
<i>E.coli</i>	3(6)	5(10)	8(16)
<i>K.pneumonia</i>	1(2)	5(10)	6(12)
<i>P.mirabilis</i>	2(4)	2(4)	4(8)
<i>B.subtilis</i>	2(4)	3(6)	5(10)
<i>A.israelii</i>	0(0)	3(6)	3(6)
Total	11(20)	21(44)	23(64)

These findings are supported by different studies, which have been conducted to evaluate the bacteria that adhere to IUD, and colonize the vagina and cervix during IUD use. In one study *E.coli* was the most common microorganism (Tsanadis *et al.* , 2002 ) . In another study *E.coli* and *Streptococcus* were isolated from the cervical canal and ectocervix of women with IUD (Levsky& Herkovits, 2005).

Regarding studies performed on Arab and Muslim women, an Iraqi study found a strong association of *Klebsiella* with IUD use (14.5%) and much less association with isolation of *E.coli* and *S.aureus* in percent of 4.3% and 8.7 % respectively (Sharief,1998).

Few studies explained the isolation of these types of bacteria among the IUD users. These studies found that isolation of *E.coli* were higher among IUD users than those who use no contraceptives, also higher during 1-7 days of menstrual cycle than in pre-menstrual, and higher among women with history of UTI (Ghazal *et al.* , 2004) The effect of menstrual cycle phase on the isolation of *E.coli* or *Streptococcus* bacteria explains the presence of these types of bacteria among the non-users in our study, taking in consideration that this group has attended the clinic while conducting the study to initiate use of IUD, (usually the time of insertion is at 5-7th day of the menstrual cycle).

Table 3 shows the relationship between the duration of use IUD and positive culture. During the first year, more than 21(42%) of users had positive culture. The negative culture (no growth results) reached up to 2(4%) among the IUD users, most of them were during the first year of use. The percent of pathogenic swabs decrease with increasing duration of use.

**Table(3) The relationship between duration of IUD usage and the number of women with infections**

Duration of IUD usage	Number & percentage of women infections	Number& percentage of women uninfected	Total
< 1 year	21(42)	2(4)	23(46)
1– 4 years	6(12)	6(12)	12(24)
> 4 years	5(10)	10(20)	15(30)
Total	32(64)	18(36)	50(100)

These findings are supported by different studies, which have been showed high percentage of potentially pathogenic bacteria among IUD users during the first year after

insertion with significant decrease of this type of bacteria after 36 month of IUD (Sayeste *et al.*, 2006).

In 2005, Pál and colleagues examined 10-year-old IUDs for biofilm formation in parallel with culture. Biofilm formation, involving both coccal and bacillary forms, was detected on the surface of the IUD by scanning electron microscopy. Quantitative culture of aerobic and anaerobic bacteria showed a dominance of anaerobic bacteria in this biofilm. The authors reported that the complexity of the biofilm flora and the dominance of anaerobic bacteria on the IUDs older than 5 years was remarkable, regardless of whether the patient had BV flora in their vagina or had no symptoms or signs of genital infection. Intrauterine devices are one of the most effective, long term methods of contraception.

Table 4 shows that only 9(18%)of women in the study had no symptoms, they were almost equally . The most frequently reported symptom was the presence of abnormal vaginal discharge (a total of 50 women).

**Table(4) Symptoms of 32 women users of IUD**

Symptoms	No.	%
No symptom	9	18
Abnormal vaginal discharge	17	36
Pelvic pain	6	12
Vaginal dyspareunia	9	18
Vaginal itching	6	12
Burning	15	30
Fever	8	16

It was found that the pattern of IUD-associated infections included acute endometritis, pelvic inflammatory disease, and unilateral ovarian abscess. Aerobic and anaerobic flora of female genital tract account for most of IUD infections. Another study evaluated the different factors affecting flora of the female genital tract such as pH, estrogen, and concentration of the mucous, which depends on the female age(Yen *et al.*.,2003).

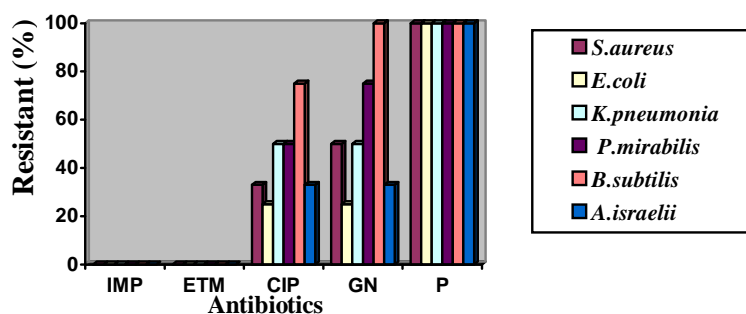
Concerning contraceptive methods, the association between IUD use and BV has been unclear. Some studies reported an increase risk of BV in IUD users, with the explanation that IUD might change the vaginal flora in favor of the growth of bacteria associated with BV and should be screened prior to IUD insertion , but others reported no association (Bartalena *et al.*.,2007 ) .

It was found that IUD use significantly increased the risk of BV when compared with other methods, mostly hormonal contraceptives, the methods undisputedly identified to decrease the risk of BV, although no satisfactory mechanism has been described ( Aitken *et al.* , 2008) . Therefore, when compared with tubal ligation and non contraceptive users, methods that are likely to have no risk for changing vaginal flora, IUD seemed to increase the risk of BV but not significantly. This possibly reveals no actual association between IUD and BV, otherwise the limitations of the present cross-sectional study need further well controlled studies to explain the issue( Levsky & Herkovits, 2005).Antibiotic resistance of bacteria becomes a world wide problem: The prevalence of resistance bacteria may lead to selection of either non-effective or expensive drugs prolonged treatment and greater risk of death(Mouton, 2002).Susceptibility of the bacterial isolates were performed against 5 different antibiotics, and the inhibition zones of them to each isolate were measured and compared with their active values against the microorganisms.The antibiotic activities against the bacterial isolates were ranging from the complete sensitive up to the

complete resistance. The results showed that all bacterial species isolates were sensitive to Impenem & Meropenem and resistant to Penicillin .

**Table (5) Antibiotic sensitivity test of bacterial species**

Antibiotic Bacterial species	Impenem		Ertapenem		Ciprofloxacin		Gentamycin		Penicillin	
	Sensitive No.(%)	Resistant No(%)	Sensitive No.(%)	Resistant No(%)	Sensitive No.(%)	Resistant No(%)	Sensitive No.(%)	Resistant No(%)	Sensitive No.(%)	Resistant No(%)
<i>S.aureus</i>	5(83.3)	0(00.0)	6(100)	0(00.0)	2(33.3)	4(66.6)	3(50)	3(50)	0(00.0)	5(83.3)
<i>E.coli</i>	8(100)	0(00.0)	8(100)	0(00.0)	2(25)	6(75)	6(75)	2(25)	0(00.0)	8(100)
<i>K.pneumonia</i>	6(100)	0(00.0)	6(100)	0(00.0)	3(50)	3(50)	3(50)	3(50)	0(00.0)	6(100)
<i>P.mirabilis</i>	3(75)	0(00.0)	4(100)	0(00.0)	2(50)	2(50)	4(100)	0(00.0)	0(00.0)	3(75)
<i>B.subtilis</i>	5(100)	0(00.0)	5(100)	0(00.0)	4(75)	1(25)	0(00.0)	5(100)	0(00.0)	5(100)
<i>A.israelii</i>	3(100)	0(00.0)	3(100)	0(00.0)	1(33.3)	2(66.6)	2(66.6)	1(33.3)	0(00.0)	3(100)



**Figure -1- Antibiotic resistant of bacterial species**

The results it appeared that most of these isolates resisted the Penicillin . The high resistance rates of Gram-positive and Gram-negative species to Penicillin . Increasing of bacterial resistance rates to this group of antibiotics may come from either production of  $\beta$ -lactamase enzyme that had the ability to destroy the  $\beta$  -lactam ring in these antibiotics, also it may come from minimizing the interaction of antibiotics with target site (Penicillin Binding Proteins) which are surface proteins responsible for cell wall synthesis (Bradford , 2001).

Augmentin had more activity than other penicillins due to its containing of clavulanic acid, which acts on inhibiting of  $\beta$ -lactamase enzymes, and causing increasing the spectrum of amoxicillin against gram-positive and gram-negative bacteria. Many researchers illustrated the higher activity of imipenem (related to Carbapenem group) against Gram-positive and Gram-negative bacteria(Bradley *et al* .,2003).The Carbapenem (Impenem &Ertapenem ) antibiotic is a beta- lactam antibiotic characterized by its ultrabroad spectrum of activity against clinically important aerobic gram – positive and gram-negative bacteria .Its wide antibacterial spectrum and great beta-lactamase for stability make Carbenem an a monotherapy in serious bacterial infections . Clinical studies also report a significantly higher success rate for Carbapenems over other antimicrobials (Kathryn *et al* ., 2005) .

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