

Pathogenic Cervical Colonization by Aerobic Gram Positive Cocci in Association with Using Two Different Methods of Contraception⁺

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Abstract :

Intra-uterine contraceptive device (IUCD) is popular and widely used method of contraception. So to assess the pathogenic cervical colonization by aerobic Gram positive cocci and whether it is due to the method of contraception used and to study the differences (if any) in the type and percentage of bacteria isolated in both groups, then study the antibiotic susceptibility of the pathogenic bacteria isolated.

A total of 300 cervical swabs were collected from 100 women visit the Family Planning Clinic in Al-Kadhymia Teaching Hospital for follow IUCD fitting and from 50 women used oral contraceptives (two swabs from each). The swabs were cultured and the results showed isolation of the same bacterial species from both groups, with isolation of *Staphylococcus aureus* from women using IUCD only.No statistically significant differences between the two studied groups regarding the types of bacteria isolated or quantity of isolation .The antibiotic susceptibility reveals high sensitivity of *Staphylococcus aureus* isolates to Cloxacillin (85.7%), so Cloxacillin could be considered as a drug of choice. Group B streptococci were highly susceptible to Ampicillin, Penicillin and the first generation of Cephalosporins. So women using IUD requires regular follow up, clinical examination, counseling and further investigation if required .

المستخلص:

إن استعمال موانع الحمل الرحمية من الطرق الشائعة الاستعمال كمانع للحمل لدى النساء ولغرض معرفة التواجد المرضي للمكورات الهوائية الموجبة لصبغة كرام في عنق الرحم ومدى ارتباطه بنوع مانع الحمل المستخدم ولدراسة الفروقات (إن وجدت) في نسبة و نوع الجراثيم المعزولة من المجموعتين ومن ثم دراسة نمط الحساسية للمضادات الجرثومية

تم جمع 300 مسحة من عنق الرحم أخذت من 100 امرأة مراجعة لعيادة تنظيم الأسرة في مستشفى الكاظمية التعليمي لغرض متابعة حالة مانع الحمل الرحمي ومن 50 امرأة تستخدم حبوب لمنع الحمل (مسحنتين من كل امرأة) وقد تم زرع المسحات وأظهرت النتائج عزل نفس الأنواع الجرثومية من المسحات المأخوذة من المجموعتين و عزل المكورات العنقودية الذهبية من المسحات الخاصة بالنساء المستخدمات لمانع الحمل الرحمي فقط كذلك عدم وجود فرق احصائي معنوي في نسبة ونوع البكتيريا المعزولة من المجموعتين. أظهرت نتائج فحص الحساسية للمضادات الحيوية حساسية المكورات العنقودية الذهبية بنسبة عالية لـ Cloxacillin وعليه يمكن اعتبار الـ Cloxacillin هو العلاج المفضل لمثل هذه الحالات أما المكورات المسببة لمجموعة B فقد كانت حساسة لمجموعة واسعة من المضادات (الأمبيسيلين ، البنسيلين ، والجيل الأول من السيفالوسبورين).

Introduction :

The population growth and use of contraceptives are topics of concern in all parts of the world [1]. Intra-uterine contraceptive device (IUCD) is commonly used method in family planning, they are small flexible device that are inserted through the

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cervical canal into the uterus by physician [2]. The mechanism of action of IUCD is thought to involve either spermicidal or inhibiting effects of sperm capacitating and transport [2].

Reproductive tract infection is one of the major complications caused by prolonged usage of IUCD [3]. IUCD offers no protection from sexually transmitted diseases (STDs) and there is a slight increase in the risk of pelvic inflammatory disease (PID) in the first weeks and months of insertion [4].

Oral contraceptives (OC), the most widely used contraceptive method worldwide. These pills contain hormones that arrest the ovulation process thus preventing pregnancy [2].

Materials and methods :

The research comprised of the study subjects (n=100) using IUCD for variable period and the control subjects (n=50) were women using OC.

The subjects were chosen according to the following criteria: (last coitus \geq 3 days, duration of contraception more than one month, not during menstrual cycle and not on antibiotic use).

During pelvic examination the cervical swabs were taken in duplicate by the gynecologist at the Family Planning Clinic at Al-Kadhymia Teaching Hospital.

The swabs were examined by:

1. Gram stain (direct examination) : to detect presence of pus and bacterial cells as shape ,Gram's stain reaction and arrangements..

2. Culture : the swabs were cultured on two plates of blood agar; one incubated aerobically and the second incubated under CO₂ and one chocolate agar plate incubated under CO₂ at 37 °C for 48 hours. Gram's stain and other differential and diagnostic tests were performed according to [5].

3. Identification of *Staphylococcus species* (staph.) was done by:

- a. Catalase test.
- b. Coagulase test.
- c. Growth on mannitol salt agar.
- d. Using Novobiocin differentiation disc.

4. Identification of β -hemolytic streptococci (strept.) by:

- a.Culture characteristics.
- b.Lancefield grouping.

5. Identification of α -hemolytic streptococci by:

- a.Culture characteristics.
- b.Using Optochin differentiation disc.

6. Antibiotic susceptibility : was done using disc diffusion method on Mueller-Hinton agar for *Staphylococcus species* and on blood agar for group B Streptococci.

The plates were incubated at 37 °C for 24 hours. The zones of inhibition were then measured and recorded according to [6].

The types of antibiotic used and their concentration were: amoxicillin 25 µg, ampicillin 25 µg, cephalexin 30 µg, cephalothin 1 µg, cefotaxime 30 µg, erythromycin 15 µg, gentamicin 10 µg, penicillin G 10 units, rifampicin 30 µg, and cloxacillin 5 µg.

7. Statistical analysis :

Statistical analysis were conducted to describe the relationship of different variables with each other .Chi-Square and Fisher test were used for quantitative and descriptive data to reflect on the level of significance of difference and / or the association between variables.

Results :

The characteristics of the two studied groups were listed in table 1.

Table 1: Characteristics of women in the two studied groups

Character	IUCD n=100	OC n=50
Women age		
mean±SE	33.09 ± 0.62	29.65 ± 1.1
Range	20 - 53	20 - 40
Contraception duration (years)		
mean±SE	1.83 ± 0.15	1.86 ± 0.16
Range	0.4 - 5	0.4 - 5

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The results revealed that the mean age of IUCD users was significantly different ($p < 0.05$) from the mean age of the OC users. Both IUCD and OC users were comparable in contraception duration. The aerobic Gram positive cocci isolated from cervical swabs of women in both groups were listed in table 2.

Table 2: Comparison between types of bacteria isolated from cervical swabs by Chi-square and Fisher test.

Bacterial species	IUCD n=100		OC n=50		Level of significance (P- value)
	No.	%	No.	%	
<i>Staph. aureus</i>	7	7	--	--	NS
<i>Coagulase negative staphylococci</i>					
<i>Staph. epidermidis</i>	2	2	2	4	NS
<i>Staph. saprophyticus</i>	2	2	2	4	NS
<i>Viridance streptococci</i>	20	20	13	26	0.042
<i>Group B streptococci (Strept. agalactiae)</i>	14	14	1	2	0.027

n = number of sample , no. = number , % = percentage

The results showed isolation of *Staph. aureus* from women using IUCD only (7%). *Staph. epidermidis* and *Staph saprophyticus* were isolated from 2% of women using IUCD and 4% from women taking OC while the isolation percentage of *Viridance streptococci* was higher in women using IUCD (20%) than in women taking OC (26%). Group B streptococci isolated from women using IUCD 14% versus 2% from women using OC.

There was no statistically significant differences between the two studied groups regarding the quantitative and qualitative types of bacteria presenting in

cervix and there were no differences regarding the bacterial species or quantity between women with different duration of using contraception. The results of antibiotic susceptibility for *Staph. aureus* and group B streptococci isolated from women using IUCD were listed in table 3.

Table 3: Percentage of sensitivity to different antibiotic discs

Antibiotics	<i>Staph. aureus</i>	<i>Group B Streptococci</i>
	%	%
Ampicillin	71.4	92.8
Amoxicillin	42.8	78.5
Cephalexin	71.4	85.7
Cephalothin	71.4	85.7
Cefotaxime	71.4	85.7
Erythromycin	57.1	78.5
Gentamicin	71.4	28.5
Penicillin G	10.2	85.7
Rifampicin	42.8	78.5
Cloxacillin	85.7	35.7

Staph. aureus isolates 89.8% were resistant to penicillin G and 85.7% were sensitive to cloxacillin. Group B streptococci were sensitive to several antibiotics, ampicillin, penicillin and cephalosporins, and 21.5% of the isolates were resistant to erythromycin.

Discussion :

IUCD is probably the second most commonly used reliable, reversible method of preventing pregnancy with more than 60 million users worldwide ,only oral hormonal contraceptive are used more frequently [7].

IUCD when used by the right person, it is extremely effective, safe, easily reversible and convenient but tends to have same side effects [3].

Female reproductive tract infection originates in lower reproductive tract (external genitalis, vagina and cervix). In the absence of early treatment, they can

spread to the upper genital tract (uterus, fallopian tubes, ovaries) so we can confide that the normal vaginal microflora is the endogenous source for infections [8].

In WHO study suggest that there are two types of PID among IUCD users, the first one related to STDs and the second type is transient PID, which is related primarily to ascent of endogenous vaginal or cervical infection during IUCD insertion [4].

The IUCD increases the myometrial activity which may increase the ascent of bacteria to fallopian tubes [8].

The OC users might have a protective effect against PID due to thickening of cervical mucus leading to reduced ascent of organisms, some types of immunogenic protection, and decrease in menstrual flow resulting in less favorable environment for bacterial growth [9].

OC provides no protection against lower genital tract infections and only marginally reduce the risk of PID and severity of tubal inflammation [10].

The ages of IUCD users were significantly higher than the ages of OC users and this may be due to the fact that OC pills are not usually prescribed for older women because of increased risk of vascular disorders ,therefore, IUCD is preferred by older women and their doctors [11] .

Women using IUCD had significantly more Gram positive cocci colonized in their cervix than women using OC, and this result was confirmed by [12] .

Group B streptococci had higher carriage rate in women using IUCD than in women using OC and this result was also explained by [12] , who said that the insertion of IUCD had double the carriage rate of group B streptococci and the tails of IUCD may aid entrance of organisms to upper genital tract. The relative risk for IUCD users is 3X than of non users and the most increase in risk is in the first four months of use, with no increase in risk thereafter and this confirmed our results that there was no differences in cervical flora between women with different duration of using contraception [13] . Group B streptococci frequently colonize the lower female genital tract with an asymptomatic carriage rate in pregnancy of 5-30%, this rate depends on maternal age, gravidity and geographic variation. Vaginal carriage is asymptomatic and intermittent with spontaneous clearing in approximately 30% and recolonization in about 10% of women. Adverse perinatal outcomes associated with group B streptococci colonization include urinary tract infection, intrauterine infection, premature rupture of membrane, preterm delivery and postpartum endometritis [14] .

The results of antimicrobial susceptibility could be helpful in choosing the suitable antibiotic in empirical therapy by the clinician.

It was found that 89.8% of *Staph. aureus* isolates were resistant to penicillin G due to its ability to produce β -lactamase (penicillinase enzyme) [15] , while 85.7% of the isolates were sensitive to cloxacillin, so this could be considered as the drug of choice in these cases, these results were in agreement with [15] .

Group B streptococci were found to be highly susceptible to penicillin's group and first generation of cephalosporins and this result was in agreement with [16]

,while 21.5% of the isolates were resistant to erythromycin and this resistance was conferred either by methylases that modify the ribosomal target of macrolides or by pumps that efflux these antibiotics [17] .

It is generally recognized that using of different methods of contraception result only in quantitative (not significant) but not qualitative difference in cervical colonization.

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