

## **Molecular Detection of CopA Gene Belongs to Escherichia coli isolated from women Using Metallic Intrauterine Contraceptive Device**

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**Abstract:** Women's contraceptives are a highly effective method of preventing conception, with success rates of up to 99 percent . They are divided into two types: the intrauterine contraceptive device (IUCD) and the oral contraceptive pill (OCP) . Contraceptives are thought to influence the amount and location of typical floral bacteria in the female reproductive system .

The rise in Vaginitis infection cases, particularly among IUCD users, could be related to a variety of factors, including a decrease in the number and prevalence of Lactobacillus spp. bacteria in this group of women, as well as a significant increase in other bacterial kinds such as Escherichia coli, which rose several times when compared to the control group, as well as cervix injury. Escherichia coli is one of the causes of vaginitis, and it is one of a group of aerobic bacteria that infect the female reproductive system and that are transmitted through sexual contact and can infect other parts of the body such as the intestines and cause many diseases. copA copper resistance gene present in E.coli. The goals of this study is to investigate the prevalence of Escherichia coli in two groups of women (one group use intrauterine contraceptive device IUCD and the other group don't use any type of contraceptive) with bacterial vaginosis infection and the role of the copA gene from E.coli in women who use contraceptives and have inflammation by conventional and molecular methods and to determining the copA gene of these isolate. Clinically suspected women with symptoms and signs of bacterial vaginosis. Who recruited into out-patient clinics of Gynecology and Obstetrics, in Al-Imammian AL\_Kadhmain Teaching Hospital\AL\_ Elweya Maternity Hospital\Kamal Al\_Samarrai Hospital\Ibn Al\_Baladi Hospital\Fatima Al\_ Zahraa Hospital ;enrolled in this cross-sectional investigation.

Two high vaginal swabs from each women were used , Vaginal swabs were taken by gynecologic .The first swab was utilized for a direct wet-mount smear, smell test, and Gram stain smear, while the second was cultured for additional microbiological assessments. Out of 200 high vaginal swabs were culture positive on deferent media showed that in G1 5(5%)E.coli while In G2 8(8%)E.coli and there is no Significance differences .

While PCR targeting (copA gene) revealed this gene was present in all E.coli isolated from G1 and present in 6 isolated E.coli from G2 ,while absence in 2 isolated E.coli from G2 with a product size of approximated 765 bp.

All swabs from G2 were examined to detect copper in uterus by absorption method and all the results were negative .

The conclusion That there is no copper released from contraceptives, and the evidence is the presence of the gene in the two groups, and perhaps there are other factors that have to do with the presence of bacteria in the women of the second group.

**Keywords:** Escherichia .coli, intrauterine contraceptive device (IUCD), copA gene.

**Introduction:** Vaginal flora is a significant element in defending the vagina from microbial species and invading diseases, as it is one of the vagina's defense systems, with lactic acid bacteria being the most important of these sorts (Lactobacillus spp.)(Abou Chacra and Fenollar, 2021) . Women's contraceptives are a highly effective method of preventing conception, with success rates of up to 99 percent (Birgisson et al., 2015). They are divided into two types: the intrauterine contraceptive device (IUCD) and the oral contraceptive pill (OCP) (Dakhal, 2020). Contraceptives are thought to influence the amount and location of typical floral bacteria in the female reproductive system (Sirota, Zarek and Segars, 2014).

The rise in Vaginitis infection cases, particularly among IUCD users, could be related to a variety of factors, including a decrease in the number and prevalence of Lactobacillus spp. bacteria in this group of women, as well as a significant increase in other bacterial kinds such as E. coli, which rose several times when compared to the control group, as well as cervix injury (Calzolari et al., 2000). Many studies have shown that OCP has no effect on (Vaginal bacterial flora) when compared to the effect of IUCD, which is associated to an increase in E. coli and most forms of Gram negative bacteria colonizing the vagina and thereby affecting the spread of Lactobacillus spp. (Nijris, Hassen and Al-Douri, 2020).

Many studies have linked the use of an IUCD to the development of BV. It's been suggested that the IUCD's tail encourages the growth of germs linked to BV in the vaginal canal (Baeyertz and Hartfield, 1997). Furthermore, the presence of BV after IUCD implantation has been linked to an increased risk of problems in the first three months of use, including Pelvic Inflammatory Disease (PID) (Carr and Espey, 2013). The findings of a study on the influence of contraceptives in women with bacterial vaginosis, which revealed a decrease in Lactobacillus spp. and a large increase in Escherichia coli (Babu et al., 2017). The explanation for this shift in bacterial kinds among Copper - IUCD users is may be due to the high levels of copper ions present in this type of contraception, which are poisonous and lethal to some bacterial species, particularly Lactobacillus spp., while E. coli has a heavy metals resistance gene (Nijris, Hassen and Al-Douri, 2020).

Cue and Cus, two chromosomally encoded copper resistance mechanisms, have been discovered in E. coli. Under aerobic and anaerobic settings, the cue (for Cu efflux) system was discovered to be the major mechanism causing copper resistance in E. coli (Å and Grass, 2003).

CueR, a copper-responsive metallo regulatory, is present in this system. CueR has been found to control the expression of two genes, cueO and copA (Rademacher and Masepohl, 2012). Copper and silver ions both stimulate the expression of the latter gene (Jakubowicz et al.,

2010) . Copper's dual nature provides a significant obstacle to living beings. To precisely manage cellular copper concentration, they must involve many complicated processes. Copper homeostasis necessitates a complex balancing act between giving vital nutrients and avoiding fatal overabundance (Horn and Wittung-Stafshede, 2021).

## **Objectives and Methods**

### **Setting of Study and population:**

The current investigation involved high vaginal specimens which were collected from different hospitals in Baghdad, Iraq, during the period from February 2021 to April 2021; these hospitals are frequently serves as a major referral center for the patients from Baghdad and its suburb, in addition to patients from other governorates, those hospitals included (AL-Imammian AL-Kadhmain teaching hospital\AL\_ Elweya Maternity Hospital\Kamal Al\_Samarrai Hospital\Ibn Al\_Baladi Hospital\Fatima Al\_Zahraa Hospital .

This cross-sectional study was carried out in a total of 200 women who were clinically suspected to have vaginosis infection; during the period from February 2021 to April 2021 .The entry points for these participants were Obstetrics & Gynecology wards; in addition to outpatients' visitors to obstetrics and gynecology department .

Women under treatment with antibiotics, Pregnant women , women with urinary tract infection , Diabetic patient , women used frequent douching or using vaginal Deodorants , Immunocompromised patients , HIV infected pts and Early menopause, are all excluded. Demographic and clinical characteristics from cases were collected using a structured questionnaire, other clinical information was recovering from the patient records. The current study has been approved by the College of Medicine Al-Nahrain University's ethics committee and is being carried out in the department of medical microbiology.

### **Samples collection and processing**

Two swab samples were taken from each woman. The first swab was utilized for a Gram stain smear, while the second was cultured for additional microbiological assessments ( cultured on different media , IMViC TEST and Api 20E ).

. The growth of E.coli placed in phosphate-buffered saline (PBS) tube then used for DNA extraction, this DNA templet furthered used in molecular detection of E.coli based on specific primer sequence.

### **DNA extraction and PCR amplification Protocol**

In this study, the high vaginal swabs from patients were subjected to DNA extraction using Gene aid Presto™ Mini gDNA Bacteria Kit (USA); according to protocols recommended by manufacturer, the sequences of primers of resistand gene (copA gene) provided from (Bio Corp Canada) as described in table (1). Amplification was as follows: one cycle in 94°C for 3 minutes followed by 38 cycles of 94°C for 30 sec , 66°C for 30 sec, and 72°C for 40 sec, terminating in 72°C for 7 min. The PCR products were confirmed by gel electrophoresis. A molecular marker (1-kb DNA ladder; Bioneer) was run concurrently.

Table (1): primers sequences with their amplicon size Base pair (bp)

Target gene	Primer sequence (5'-3')	Product size	Reference
<b>copA-F</b>	5`- GGTGCTGATCATCGCCTG -3`	<b>765bp</b>	(Besaury et al., 2013)
<b>copA-R</b>	5`- GGGCGTCGTTGATACCGT -3`		

### Statistical analysis

The results are expressed as a percentage only.

### Results:

#### Isolation and identification of Escherichia coli by conventional methods

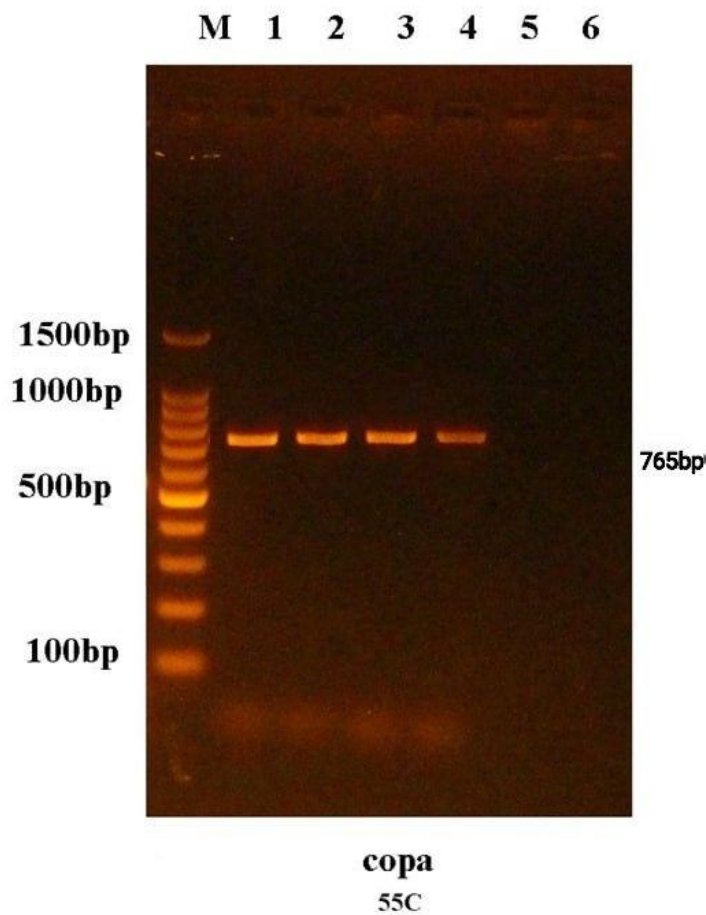
Vaginal swabs were implanted on the following media: Blood agar , EMB agar and MacConky agar The following examinations were performed to confirm the diagnosis: Oxidase test, Sugar fermentation test and API 20 strip . The results of the initial culture showed that there are 13(6.5%) out of 200 samples [ 5 (38.5%) from G1 and 8(61.5%) from G2 ] gave characteristics Escherichia coli ; as shown in table (2)

Table (2): Distribution of sample study according to results Culture

Type of contraceptive	E. coli		Total	P. value
	Negative	Positive		
don't use	95	5	100	0.389 NS
	50.8%	38.5%	50%	
used IUCD	92	8	100	
	49.2%	61.5%	50%	
Total	187	13	200	
	100.0%	100.0%	100.0%	

#### PCR for detection of resistant gene (copA gene)

Conventional PCR was done for the amplification of (copA) gene which is a resistant gene; by used specific primer sequences. The results showed that, this gene was present in 5 high vaginal swabs from G1 and 6 from G2 samples with a PCR product size of approximated 765 bp , while the remaining samples of the patients showed negative results for the presence of this gene (Figure 1).



**Figure (1):** Results of the amplification of CopA gene of *Escherichia coli* samples were fractionated on 1.5% agarose gel electrophoresis at 70 volts for 30 min, stained with Eth.Br. M: 100bp ladder marker. Lanes 1-6 resemble 765bp PCR products.

#### Discussions:

Bacterial vaginosis caused by many aerobic bacterial vaginosis. Microscopic examination, culture and biochemical tests are used for diagnosis of aerobic bacterial vaginosis. Table (1),

**Table (1) Microorganism Distribution on study groups.**

Type of contraceptive	VaginalSwab										Total	P. value
	No growth	lactobacillus	Staph. aureus	Staph. epidermitis	Candida	Strep.agalactica	mix growth	E.coli	Klebsialla	Garderenella		
Group1	22	23	7	16	15	6	4	5	1	1	100	0.000**
	37.9%	74.2%	23.3%	66.7%	78.9%	37.5%	57.1%	38.5%	100.0%	100.0%	50.0%	

			3 %		%		1 %					
Group2	36	8	2 3	8	4	10	3	8	0	0	100	
	62. 1%	25.8 %	7 6. 7 %	33.3 %	21 .1 %	62.5%	4 2. 9 %	61. 5%	0.0%	0.0%	50.0 %	
Total	58	31	3 0	24	19	16	7	13	1	1	200	
	10 0.0 %	100. 0%	1 0 0. 0 %	100. 0%	10 0. 0 %	100.0%	1 0 0. 0 %	100 .0%	100. 0%	100.0%	100. 0%	
**means High significance differences (P ≤0.001)												

Mix growth mean:

(Candida with Strep.agalactiea)

(Staph.aureus with Lactobacillus)

(Lactobacillus with Strep.agalactiea )

it is documented that the predominant of aerobic bacterial vaginosis was Lactobacillus 23(23%) from G1 and 8(8%) from G2 (This indicates the presence of inhibitory effects on the growth of lactobacillus bacteria due to the presence of contraceptives ) and the lower ratio than above was belong Staph. aureus was 7(7%) from G1 and 23(23%) from G2 and the lower ratio than above was Staph.epidermidis , candida , Srep.agalactia , mix growth ,E.coli 5(5%) from G1 and 8(8%) from G2 , Klebsiella ,Garderenella . Lactobacillus spp. produced several antimicrobial factors like H<sub>2</sub>O<sub>2</sub> and bacteriocin which gave ability to adhere on vaginal epithelial cell and prevent colonization of bacterial vaginosis (Borges, Silva and Teixeira, 2013).Donders, 2017 proved that vaginosis includes the presence of immature epithelial cells, Lactobacillus spp. and aerobic bacterial with sever inflammation due to increase the number of WBCs. The lack of estrogen played a role in the growth of aerobic bacterial vaginosis (Donders et al., 2017). (This is consistent with the current study in group 1 study ) Bitew et al., 2017 (Bitew et al., 2017).Some bacterial vaginosis was studied like E. coli which was recorded as the predominant 43(28.5%) and this disagree with the present data. Regarding Superti et al., 2020 work on vaginal discharge and its association with aerobic bacterial vaginosis, he found other types of bacteria such as Streptococcus spp group B, S. aureus in addition to E.coli .As an important aerobic bacterial vaginosis related with trimester pregnancy (Kaambo et al., 2018). This present results disagree with Samia S, 2012 (Badwi, 2016) .who found that S. aureus are the common causative agent in asymptomatic lower genital tract infection followed by E.coli . In Lybia, Al- Al-Mousawi, et al., 2006 was

recorded *S. aureus* was the dominant which colonized vaginal mucosa and causes toxic shock syndrome (Parsonnet et al., 2005) .

Razzak et al.,2011 (21) reported a high rate of bacterial vaginosis caused by Gram negative bacteria as 12(16.2%) and *S. aureus* was 14(18.9%) this study disagree with the current results. *E. coli* was a causative agent of vaginosis because *E. coli* is a normal flora in gastric tract and transferred to genital tract and causes bacterial vaginosis. *E. coli* ferment lactose to lactic acid, therefore it may be affected by the amount of glycogen, also washing under wears with strong detergent may be affect normal flora, poor body hygiene especially when using the toilet, and finally antibiotics may lead to vaginosis microflora in vagina and enhance opportunistic bacteria.( We note the proportions of bacteria isolated from the two groups are close, where the first group 63 bacterial isolates and the second group 60 isolates, which indicates that there is no effect of contraceptives on the number of isolated bacteria, but the difference is the number of bacteria types )

In this study, conventional PCR were used for detection resistant gene of *E.coli*, as a virulence factor *copA* gene with genome size 765pb as figure (1).

In this study, the isolates showed the prevalence of *copA* gene (85%) in *E. coli* isolates and the absence of the gene in 15 (15%) of *E. coli* isolates from total 13 isolated *E.coli*. The results of *Escherichia coli* consisted of 5 (5%) G1 *copA* genes and 6 (6%) G2. There is no difference between the two groups in determining the gene, which indicates that there is no effect of the contraceptive in pregnancy on increasing the rate of bacterial infection. some factors like pH, temperature, incubation time, culture media have an amount effect on the volume of *copA* gene. In spite of detection of the *copA* of *E.coli* isolate the absence of gene may be explained by mutation of the gene or lack of its expression in this isolate (Petersen and Møller, 2000).

All vaginal swabs were examend by atomic absorption spectrophotometer (AA700) to detect the copper but all the result was negative , below detection limit (BDL) the reader was part per pillions .The limit of detection for different instrument varies. Some has it as 0.001 mg/L, others as 0.01 mg/L. Copper was not identified in the swabs, which indicates that copper was not released from contraceptives This indicates the quality of the contraceptive industry . It requires more studies of other *E.coli* genes, increasing the number of samples and adopting modern diagnostic techniques.

## CONCLUSION

The prevalence of *E.coli* in the current study was 5(5%) in G1 and 8(8%) of G2 reiterate the essential diagnosed and follow-up of this bacterial infection particularly in women. All of the isolate from G1 in this study showed presence of *copA* gene and 6 isolate from G2 while the gene absence in 2 isolate from G2 may be from the effect of IUCD or mutation or there is no expression of the gene in these samples. And as a result? There is no effect of contraceptives, perhaps due to the accuracy and safety of manufacture, and we recommend more study on this gene or others.



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