

Mycoplasma hominis Detection in Females Suffering from Vaginitis Who Are Using a Contraceptive Device

Muhannad Abdullah Khalaf¹, Mustafa Mahir Khudur²

¹ Ph.D. Medical Microbiology, Kirkuk health directorate, Kirkuk, Iraq

² M.Sc. Medical Microbiology, Kirkuk health directorate, Kirkuk, Iraq

Abstract

This hospital based study was done in Kirkuk city from June to November 2021. In our study, it was aimed to investigate the frequency of *M. hominis* in women who use an intrauterine contraceptive device, and to compare that incidence with the incidence of *Mycoplasma hominis* in women who use other forms of contraception. Deep endocervical swabs were collected from 100 fertile women who attended the gynecology unit in Gynecological and Pediatric Hospital in Kirkuk city (50 of them using IUCD (cases) and 50 using other methods of contraception as barrier or hormonal as control group). For each patient three cervicovaginal samples were taken by swabs for microbiological study. The following items were included in the collection of vaginal swabs: Only a portion of the swab package had been opened. Two- The swab was gently rotated for 10 to 30 seconds after it had been carefully inserted into the vagina about two inches (5 cm) past the introitus. As soon as the swab touched the vaginal walls and moisture, it was drawn away from the skin without making contact with the skin. In accordance with standard operating procedures, the colonies that had grown on the media were harvested, purified, and identified. Smears were prepared from bacterial colonies, stained with Gram stain, and examined under a light microscope for morphological characteristics. The study revealed that 80 percent of women who have used IUCD (cases) have a positive deep cervical swab for culture, compared to 24 percent of women who have used other types of contraception such as barrier or hormonal contraception (control group). *Mycoplasma hominis* (52.5 percent) was the most frequently isolated bacteria from women who had used IUCD. *Ureoplasma ureoliticum* and *E. coli* were the most frequently isolated bacteria from the control group, with 33.33 percent for each and no *Mycoplasma hominis* isolated from control women. According to the findings of the study, 50 percent of women who used IUCD with positive culture suffered from Pelvic pain, 37.5 percent suffered from abnormal vaginal discharge, 40 percent suffered from burning, 15 percent suffered from fever, 25 percent suffered from vaginal dyspareunia and vaginal itching, and 5 percent suffered from no symptoms. The study showed that majority of women used IUCD who infected with *M. hominis* were used IUCD for more than 6 years (38.1%) and 9.52% in women who used IUCD less than 1 year, while 40% of women without infection were within group below 1 year.

Conclusion: *Mycoplasma hominis* was the most frequently isolated bacteria from women who used contraceptive devices and suffered from vaginitis.

Recommendations: Additional research is needed to investigate the relationship between *Mycoplasma hominis* and pregnancy outcomes such as abortion

Keywords: *Mycoplasma*; contraceptive devices; Vaginitis

Introduction

PID can result in damaging effects to a woman's reproductive organs that can not be reversed even if PID is treated and cured. The most common method of treatment is the use of antibiotics. Since not only does *M. hominis* cause PID, usually two different types of antibiotics are prescribed in an effort to kill all the possible infectious agents (1,2). If symptoms become severe and are not immediately cared for, then hospitalization may be needed to prevent further complications. Complications with PID can critically injure reproductive organs and may cause infertility. Bacteria involved in PID can cause reproductive organ tissue to change into scar tissue resulting in infertility. It is known as pelvic inflammatory disease (PID) because it affects the female upper genital tract and is an infection disorder. It usually manifests itself as an ascending infection from the endocervix and vaginal area, resulting in endometritis, salpingitis, tubo-ovarian abscess,

parametritis, oophoritis, and/or pelvic peritonitis, among other complications (3,4). Infections caused by sexually transmitted organisms such as *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT) are among the many agents implicated in the pathology of PID (5). Mycoplasmas are microorganisms that can be found in nature as colonizers in animals and humans, and generally settle in the respiratory tract and genital system in humans. Among the species that are members of the *Mycoplasma* and *Ureaplasma* genera, there are opportunistic pathogens for humans, and some are members of the normal flora of various body parts in humans. The most frequently isolated mycoplasmas from the urogenital system are *Mycoplasma hominis* and *Ureaplasma urealyticum*. Mycoplasmas are found in the normal genital flora of many sexually active and healthy men and women. Colonization is more common in young women and in societies with low socioeconomic status(8,12,14,16,17). It has been reported that genital mycoplasmas are isolated from cervicitis, salpingitis and pelvic abscesses, and from blood during post-puerperal fever (14). Genital mycoplasmas are microorganisms found in many places, especially in the lower genital system of sexually active individuals, and isolated from the upper genital tract in patients with salpingitis(8). Isolation and identification of *M.hominis* must be done accurately and reliably in order to reveal the importance of the diseases they cause in humans. Although various special media formulas have been developed for their production, many methods have been developed, from ready-made media to polymerase chain reaction, aiming to isolate them quickly and easily due to the difficulties in their preparation and the late growth of these bacteria (10). In our study, it was aimed to investigate the frequency of *M.hominis* in women who use an intrauterine contraceptive device, and to compare that incidence with the incidence of *Mycoplasma hominis* in women who use other forms of contraception.

Materials and Methods.

This case-control, hospital based study was done in Kirkuk city f June to November 2021. Deep endocervical swabs were collected from 100 fertile women who attended the gynecology unit in Gynecological and Pediatric Hospital in Kirkuk city (50 of them using IUCD (cases) and 50 using other methods of contraception as barrier or hormonal as control group). Demographic, medical and gynecologic histories as well as information about duration of IUD use were obtained, and a pelvic examination was performed in all patients. For each patient three cervicovaginal samples were taken by swabs for microbiological study. The following items were included in the collection of vaginal swabs:

1- Only a portion of the swab package had been opened.

Two- The swab was gently rotated for 10 to 30 seconds after it had been carefully inserted into the vagina about two inches (5 cm) past the introitus.

3- As soon as the swab touched the vaginal walls and moisture, it was drawn away from the skin without making contact with the skin.

4- Swab samples were delivered to the laboratory within one hour of being collected.

5- The swab samples were cultured in blood agar and MacConkey agar for a total of 24 hours. The media were prepared and sterilized in accordance with the manufacturer's recommendations. It was necessary to carry out these tests on the prepared media after they had been solidified in order to carry out isolation, viable count determination, identification, and susceptibility testing on them.

After taking full history and clinical examination, the specimen was obtained from the endocervix with sterile cotton swab and transferred immediately into a tube containing Stuart transport medium, and quickly taken to the central laboratory where the culture was performed. It is traditional to culture mycoplasmas in a peptone-enriched beef heart infusion broth (commercially available as PPLO broth) supplemented with horse serum and yeast extract, and then to demonstrate their presence in the culture medium. A specific arginine metabolization by *Mycoplasma hominis* results in the release of ammonia and an increase in pH; therefore, the addition of a defined substance (arginine) to the culture medium causes a mycoplasma, if present, to change the pH of the culture medium. Cotton swabs were used to collect samples, which were then incubated for 24-48 hours at 36 degrees Celsius in a *Mycoplasma System Plus* (Liofilchem Bacteriology Products-Italy) kit. Using color change in the wells, it was possible to demonstrate the presence of *Mycoplasma hominis* and *Ureoplasma ureolyticum*. In accordance with standard operating procedures, the colonies that had grown on the media were harvested, purified, and identified. Smears were

prepared from bacterial colonies, stained with Gram stain, and examined under a light microscope for morphological characteristics.

Results.

Table 1 shows that 80 percent of women who have used IUCD (cases) have a positive deep cervical swab for culture, compared to 24 percent of women who have used other types of contraception such as barrier or hormonal contraception (control group)

Table 1: Results of cervical swab among the study groups.

The outcome of the vaginal swab culture	Study groups			
	Cases		Control group	
	No.	%	No.	%
+ve culture	40	40	12	12
Negative	10	10	38	38
Total	100	100	100	100

P<0.001

Table 2 shows that *Mycoplasma hominis* (52.5 percent) was the most frequently isolated bacteria from women who had used IUCD (cases), followed by *Ureoplasma ureoliticum* (20 percent) and *E. coli* (15 percent). *Ureoplasma ureoliticum* and *E. coli* were the most frequently isolated bacteria from the control group, with 33.33 percent for each and no *Mycoplasma hominis* isolated from control women.

Table 2: The distribution of pathogenic bacteria isolated from patients among the study groups.

Bacterial isolates	Study groups			
	using IUCD (cases)		(control group)	
	No.	%	No.	%
<i>Mycoplasma hominis</i>	21	52.5	0	16.67
<i>Ureoplasma ureoliticum</i>	8	20	2	33.33
<i>Escherichia coli</i>	6	15	4	8.33
<i>Gardnerella vaginalis</i>	2	5	1	16.67
<i>Staphylococcus aureus</i>	1	2.5	2	25
<i>Klebsiella</i>	2	5	3	16.67
Total	40	100	12	100

P<0.001

According to the findings of the study, 50 percent of women who used IUCD with positive culture suffered from Pelvic pain, 37.5 percent suffered from abnormal vaginal discharge, 40 percent suffered from burning, 15 percent suffered from fever, 25 percent suffered from vaginal dyspareunia and vaginal itching, and 5 percent suffered from no symptoms.

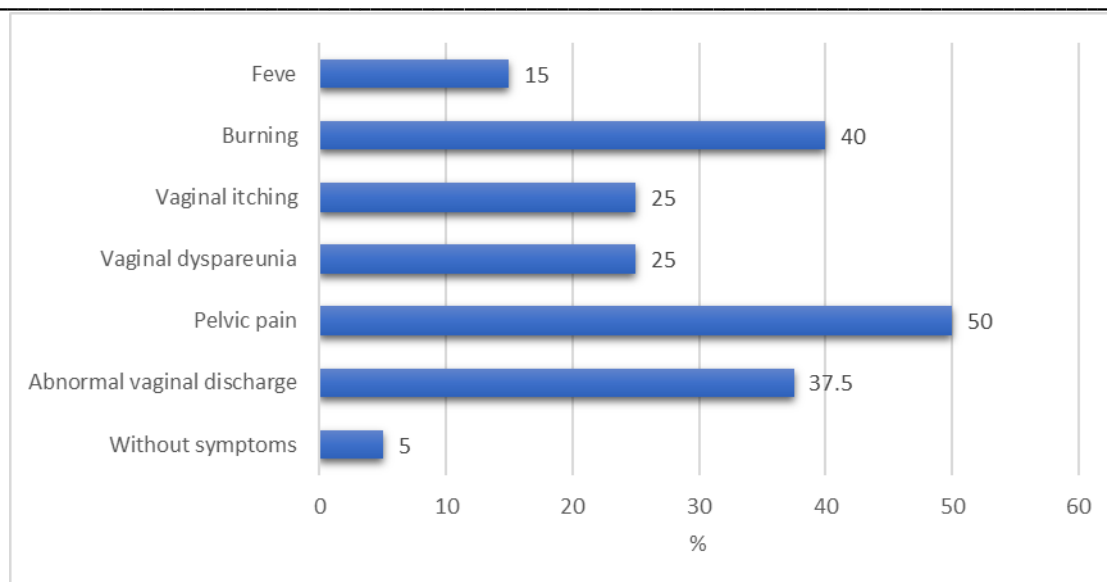


Figure 1: Distribution of IUCD women with +ve culture according to presence of symptoms of pelvic infection

The study showed that majority of women used IUCD who infected with *M. hominis* were used IUCD for more than 6 years (38.1%) and 9.52% in women who used IUCD less than 1 year, while 40% of women without infection were within group below 1 year, Figure 2

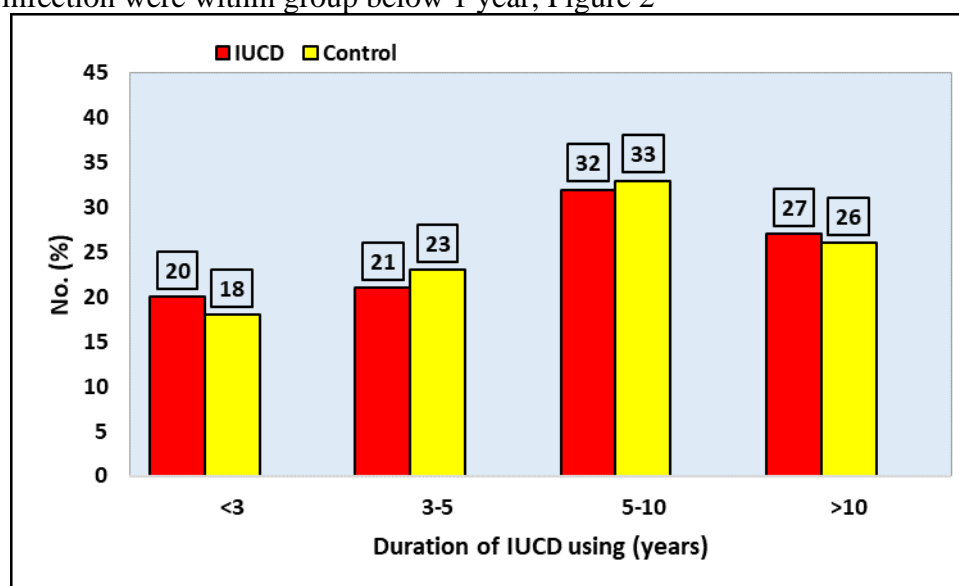


Table 2: Relation of pelvic infection with duration of IUCD using

Discussions

PID is a disease which infects a woman’s reproductive systems including the uterus and the fallopian tubes. This illness happens when bacteria, specifically *M. hominis*, travel to inner reproductive organs from their common locations of the cervix and vagina⁽⁴¹⁾. PID is more likely to occur in women who are sexually active and have many different sexual partners which increase potential exposure to infection-causing pathogens. Similar to bacterial vaginosis, PID is caused by alterations to the composition of vaginal bacterial flora. Vaginal flora can be altered by uses of douches and intrauterine apparatuses which push *M. hominis* upward into reproductive organs⁽³⁴⁾. The findings of the study revealed that *Mycoplasma hominis* (52.5 percent) was the most frequently isolated bacteria from women who had IUCD (cases), followed by *Ureoplasma ureoliticum* (20 percent) and *E. coli* (15 percent). The study also revealed that the most frequently isolated bacteria from the control group used were 33.33 percent for each of *Ureoplasma ureoliticum* and *E. coli*, and that *Mycoplasma hominis* was not isolated from the control women. The findings of other studies indicated that the bacteria *Staphylococcus aureus* may have only been organisms

causing local vaginal infection because they were not found in the endocervix, and that they may not have been responsible for the ascending upper genital tract infection (2). Dutta et al (3), on the other hand, reported that *Staphylococcus aureus* is one of several organisms that have been reported to cause pelvic infection in the literature. Other studies on vaginitis conducted in recent years revealed that micro-organisms introduced into the uterus as a result of the use of inadequately sterilized instruments were also concerned, with the most commonly implicated organisms being *Escherichia coli* and staphylococci being the most common (4,5). According to Ahmadi et al (6), the frequency of 128 bacterial isolates of vaginitis cases was determined to be 39.06 percent for *M. hominis*, 22.6 percent for *Escherichia coli*, 14.8 percent for *Klebsiella pneumoniae*, and 6.2 percent for *Lactobacillus* spp. This variation could be due to differences in methodology used in the isolation and identification of the etiologies of vaginal infections. Furthermore, environmental factors as well as differences in the actual study participants may also contribute to the discrepancy noted above (7,8). It is possible that the high rate of isolation of *Mycoplasma hominis* among women in the second age group (20-29 years) in both studied groups (women using the IUCD and control) can be attributed to increased sexual activity, as colonization with genital mycoplasma increases proportionally to the number of sexual partners and sexual activity(9), as well as to a lack of general medical care among young women (10).

It was discovered by Gump Ahmadi et al (6) that the presence of *Mycoplasma hominis* in the endocervix increased the rate of pelvic infection. This result was consistent with the findings of the previous study. A number of studies have also concluded that the risk of pelvic infection is 3-9 times higher among IUCD users than among non-users (11) compared to the general population. Initially, bacteria (particularly *Chlamydiae trachomatis* and *Neisseria gonorrhoea*) cause epithelial damage in the cervix, which then allows opportunistic entry of other organisms. The majority of cases of PID appear to be caused by ascending infection from the cervix. Considering that isolates from the upper genital tract are polymicrobial, including *Mycoplasma hominis* and anaerobes, the spread of infection to the upper genital tract may be inhibited by the use of a contraceptive device, instrumentation, and vaginal douching (12,13). Extensive research has been done over the past few decades on the relationship between the development of any upper genital tract infection immediately after IUD insertion and the length of time the IUD has been in place. However, the findings have been controversial (14,15). Despite the fact that previous studies(11,13) have found higher rates of PID immediately following the insertion of IUDs, some recent data allow for a more detailed examination of this relationship (16).

Conclusion:

Mycoplasma hominis was the most frequently isolated bacteria from women who used IUCD, and there was a statistically significant relationship between pelvic infection caused by *Mycoplasma hominis* and the length of time the women had been using IUCD.

Recommendations:

Additional research is needed to understand the relationship between bacteria and fungi other than *Mycoplasma hominis* and the IUCD in women. Investigate the relationship between *Mycoplasma hominis* and pregnancy outcomes such as abortion, preterm and post-term birth, and stillbirth. Instruct medical clinics and hospitals to send suspected cases of vaginosis for bacterial cultivation and to conclude treatment with a specific antibiotic in order to avoid blind treatment with antibiotics.

References

1. Subha S. Study of microbial flora of female genital tract in intrauterine contraceptive device users in a tertiary care hospital. *University Journal of Pre and Paraclinical Sciences*. 2017 Jan 9;2(7).
2. Deese J, Pradhan S, Goetz H, Morrison C. Contraceptive use and the risk of sexually transmitted infection: systematic review and current perspectives. *Open Access Journal of Contraception*. 2018;9:91.
3. Dutta S, Sengupta P, Izuka E, Menuba I, Jegasothy R, Nwagha U. Staphylococcal infections and infertility: mechanisms and management. *Molecular and Cellular Biochemistry*. 2020 Jul 20:1-6.

4. Ricci S, De Giorgi S, Lazzeri E, Luddi A, Rossi S, Piomboni P, De Leo V, Pozzi G. Impact of asymptomatic genital tract infections on in vitro fertilization (IVF) outcome. *PloS one*. 2018 Nov 16;13(11):e0207684.
5. Longdoh NA, Gregory HE, Djeumako WA, Nguedia AJ, Francois-Xavier MK, Tebit KE. The Occurrence and Antimicrobial Susceptibility Patterns of *Mycoplasma hominis* and *Ureaplasma urealyticum* in Pregnant Women in Three District Hospitals in Douala, Cameroon. *Journal of Advances in Medicine and Medical Research*. 2018 Nov 1:1-1.
6. Ahmadi MH, Mirsalehian A, Bahador A. Prevalence of urogenital mycoplasmas in Iran and their effects on fertility potential: a systematic review and meta-analysis. *Iranian journal of public health*. 2016 Apr;45(4):409.
7. Choi JB, Lee SJ, Lee MK, Lee SJ, Park DC, Kim HY, Lee DS, Choe HS. Prevalence and antimicrobial susceptibility of *Ureaplasma* spp. and *Mycoplasma hominis* in asymptomatic individuals in Korea. *Microbial Drug Resistance*. 2018 Nov 1;24(9):1391-6.
8. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine microbiota: residents, tourists, or invaders?. *Frontiers in immunology*. 2018 Mar 2;9:208.
9. Valentine-King MA, Brown MB. Antibacterial resistance in *Ureaplasma* species and *Mycoplasma hominis* isolates from urine cultures in college-aged females. *Antimicrobial agents and chemotherapy*. 2017 Oct 1;61(10).
10. Hosny AE, El-Khayat W, Kashef MT, Fakhry MN. Association between preterm labor and genitourinary tract infections caused by *Trichomonas vaginalis*, *Mycoplasma hominis*, Gram-negative bacilli, and coryneforms. *Journal of the Chinese Medical Association*. 2017 Sep 1;80(9):575-81.
11. Foran T, Butcher BE, Kovacs G, Bateson D, O'Connor V. Safety of insertion of the copper IUD and LNG-IUS in nulliparous women: a systematic review. *The European Journal of Contraception & Reproductive Health Care*. 2018 Sep 3;23(5):379-86.
12. Morrill S, Gilbert NM, Lewis AL. *Gardnerella vaginalis* as a Cause of Bacterial Vaginosis: Appraisal of the Evidence From in vivo Models. *Frontiers in cellular and infection microbiology*. 2020 Apr 24;10:168.
13. Levin G, Dior UP, Gilad R, Benshushan A, Shushan A, Rottenstreich A. Pelvic inflammatory disease among users and non-users of an intrauterine device. *Journal of Obstetrics and Gynaecology*. 2020 Feb 29:1-6.
14. Agung D, Tualeka AR, Ardyanto D. The Correlation IUDs Use Duration and Types of IUDs with Blood Curprum (Cu) Levels in Women with IUD Contraception: A Cross-Sectional Study. *Indian Journal of Public Health Research & Development*. 2019;10(12):1749-53.
15. El-sayedAbdou A, Mohamad EE, Tawfiek AM, Belbasy RE. Bacterial Infections and Biofilm Formation Associated with Intra Uterine Contraceptive Device among Females Attending Al-Glaa Teaching Hospital in Cairo. *The Egyptian Journal of Hospital Medicine*. 2018 Jan 1;70(5):882-90.
16. Tualeka AR, Dwirahmadi F, Wibowo A, Sirait FA. The use of IUD, passive smoker and the risks of cervical cancer: A cross-sectional study at female workers in Surabaya City, Indonesia. *Indian Journal of Public Health Research & Development*. 2018;9(11)