

DETECTION OF CAUSATIVE AGENTS OF SEXUALLY TRANSMITTED DISEASES IN SYMPTOMATIC INFERTILE WOMEN USING REAL TIME PCR TECHNIQUE

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ABSTRACT

Sexually transmitted infections or venereal diseases constitute a major health problem especially in developing countries, including Iraq with complications like pelvic inflammatory disease, ectopic pregnancy, cervical cancer, infertility, and congenital infections. The main goal of current study is to detect the common bacterial causes of sexually transmitted infections among symptomatic infertile women using real time PCR technique. A cross-sectional study was conducted from February 2022 to February 2023. A total of 100 vaginal swabs were collected from symptomatic infertile women with ages ranging from 20 to 49 years (mean 23.97 years \pm 6.90 SD) who attended the private health centers in Duhok, Kurdistan Region, Iraq. The vaginal swabs were collected from each patient and processed by real time PCR to detect *C. trachomatis*, *T. vaginalis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *U. urealyticum* and *U. parvum*. Out of 100 symptomatic infertile women, 68 were positive for one or more of the causative agents. The highest rate of infection was caused by *U. parvum* (50%), followed by *M. hominis* (21.0%), *U. urealyticum* (16.0%) and the lowest percentage was 1.00% for *C. trachomatis*, while all samples were negative for *N. gonorrhoeae*, *T. vaginalis* and *M. genitalium*. No significant association was found between *C. trachomatis*, *M. hominis*, *U. urealyticum* and *U. parvum* with age groups, duration of infertility and levels of education of studied symptomatic infertile women except *C. trachomatis* which showed significant association ($p=0.001$) with duration of infertility from 11-16 years. Prevalence of *M. hominis*, *U. urealyticum* and *U. parvum* in the birth canal of infertile women demands more studies because several studies in the world highlighted on the presence of association between these colonized bacteria and infertility due to the tubal damage or blockage.

KEYWORDS: Causative agents, sexually transmitted diseases, Infertile women and PCR.

1. INTRODUCTION

Sexually transmitted infections (STIs), often known as venereal diseases, are spread through sexual contact and are a major cause of illness especially in developing nations (Muralidhar, 2015). STIs are often asymptomatic, but they can cause Pelvic Inflammatory Disease (PID), Infertility, Ectopic pregnancy, Abortion, and Cervical cancer (Hasani *et al.*, 2021) as well as congenital infections (Adachi *et al.*, 2016). STDs are more frequently viewed as disease syndromes. A number of different sexually transmitted pathogens yield a common set of signs and symptoms known as a syndrome (Workowski & Bolan, 2015). In 2001, Centers for Disease Control and Prevention (CDC) mentioned *Gonorrhea* as the second most common sexually transmitted disease (STD) after Chlamydia and founded 30% of all STDs (Johnson *et al.*, 2002).

Neisseria gonorrhoeae and *Chlamydia trachomatis* are the common prevalent sexually transmitted bacteria capable of infecting men, women and neonates worldwide, especially in developing countries (Koval, 2018; Cole, 2019).

The most typical bacterial causes of STIs are *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum* (Kim *et al.*, 2015). At least once in their lifetime, 75% of women may get vaginitis. However, 90% of vaginal infections are caused by *Candida albicans*, followed by *Bacterial Vaginosis* (BV) and *T. vaginalis* (Yuan *et al.*, 2021). The diagnosis and treatment procedures for the aforementioned STDs are reserved for situations in which these infections are suspected (e.g., contact with trichomoniasis, urethral lesions, or severe dysuria and meatitis, which might suggest genital herpes) or when NGU is not responsive to recommended therapy (Kimberly and Gail,

2015). Female infertility may be attributed to a number of factors, typically divided into endocrine, vaginal, cervical, uterine, tubal, and pelvic-peritoneal factors, and although estimates vary, approximately 15-30% of cases still remain unexplained (Quaas and Dokras, 2008). Further insight into the causes of infertility is necessary to help alleviate this multifactorial burden on society. Several sexually transmitted diseases, including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, have been widely studied to understand their role in salpingitis and infertility. Additionally, several other pathogens such as *Mycoplasma genitalium*, *Trichomonas vaginalis*, and other microorganisms within the vaginal microbiome may also play roles in tubal damage and other potential causes of infertility. While screening and treatment efforts for *C. trachomatis* and *N. gonorrhoeae* have been developed to reduce the incidence of PID and subsequent infertility factors, additional data is needed to determine the role of other potential pathogens and whether early detection can prevent tubal damage (Tsevat *et al.*, 2017). Because infertile women are common in our area and data concerning their common causes are limited, therefore the current study was performed to determine common bacterial and protozoal causes of STDs among symptomatic infertile women using real time PCR technique as well as to find out their relation to some demographic characteristics.

2. METHODOLOGY

2.1. Study design and sample collection

A cross-sectional study was conducted at both private Shilan hospital and maternity hospital in Duhok city from February 2022 to February 2023. A total of 100 symptomatic infertile women suffered from vaginitis and cervicitis were enrolled in the study with ages ranging from 20-50 years, with an average 32.96 years \pm 6.96 SD. Women who received antibiotics were excluded. The endocervical swabs were collected from each patient and kept in viral transport media (VTM) for DNA extraction and RT-PCR. All participants were interviewed and a special questionnaire format was prepared and filled. The questionnaires were included age, duration of infertility, previous treatments, educational level, residence, chronic diseases with signs and symptoms.

2.2 Ethical considerations

An official approval letter from Ethic

Committee of Duhok General Health Directorate No. (211-22021-12-3) was obtained.

2.3 Molecular methods

DNA was extracted from endocervical swabs by FavorPrep™ Blood/ Cultured Cells Genomic DNA kit (FAVORGEN, South Korea) according to the instructions supplied by the kit. Bosphore® STDs Panel Bundle Kit v1 (Anatolia geneworks-Turkey) was used which detects the following pathogens: *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, *Neisseria gonorrhoea*, *Trichomonas vaginalis* and *Mycoplasma genitalium*. Four different fluorescence dyes were used: FAM, HEX, Texas RED and Cy5.

3. RESULT AND DISCUSSION

3.1 Results of multiplex real-time PCR technique:

Out of 100 symptomatic infertile women, 68 were positive for one or more of the causative agents. The highest percentage of infection (50.0%) was caused by *U. parvum*, followed by *M. hominis* (21.0%), *U. urealyticum* (16.0%) and the lowest percentage was (1.0 %) for *C. trachomatis*, while all samples were negative for *N. gonorrhoeae*, *T. vaginalis* and *M. genitalium* as shown in figure 1.

The prevalence of *C. trachomatis* infections was 1%. The results of this study were lower than those carried out in Iran (Rajabpour *et al.*, 2020) who found that 10.8% of symptomatic infertile women were positive for *C. trachomatis* and Dhawan *et al* (2014) in India found 13.5% and Shamkhi *et al* (2020) in Iraq found 13%. This discrepancy in the results can be attributed to several factors such as sample size, methodology, socio-economical level, educational levels and environmental conditions.

In the current study, no *T. vaginalis* was detected. This result disagreed with the results of others who carried out in different Iraqi governorates such as in Sulaimaniyah and Erbil cities were 1.6% and 1.66% respectively (Kadir and Fattah, 2010), in Duhok was 2.4% and 4.66% by (Al Saeed, 2011) and (Haydar and Naqid, 2022) respectively, Kirkuk (2.8%) (Kadir *et al.*, 2014), Mosul (15.5%) (Al Daheen *et al.*, 2005) as well as from studies in other countries such in Turkey (15.37%) (Yazar *et al.*, 2002), Saudi Arabia (28.1%) (Madani, 2006) and Egypt (36%) (Aboulghar *et al.*, 2009). Generally, the prevalence of *T. vaginalis* is low in Kurdistan region compared to other parts of Iraq which can be due to personal hygiene level, public health

awareness, overcrowding and geographical environment.

No *N. gonorrhoeae* infection was detected in the current study compared to other study by Haydar and Naqid (2022) who found that 1.33% of married women in Zakho city and were positive for *N. gonorrhoeae* in Baghdad, 1.66% by Al-Kaisi et al (2006). The prevalence of *N. gonorrhoeae* is very low in our area due to the religion which restrict illegal sexual activities. *U. parvum* was detected in 50% of the infertile women which is similar with the results of Ghofran Al-khafaji (2017) in Iraq who identified *U. parvum* in 29.6% from patient. Urszula and co-workers (2014) also found that 52.5% of the women were positive for *U. parvum* and 32.9% by (Choe et al., 2013). *U. parvum* has been reported to be associated with chorioamnionitis, fetal morbidity, and fetal mortality in pregnant women, but its role as an STI pathogen is unclear (Choe et al., 2013).

The rate of *M. hominis* infection was 21% in the current study which agreed with results of Majhi et al (2022) who found that 20% of sexually active women were positive, while lower than the results of Fatlawy and Abduljabbar (2019) who found that 31.12% of

the infertile women from Iraq were positive. On another hand, these results were higher than the results of Mortazavi et al (2021) in Iran who found that 7.62% of the infertile women were positive. Although high percentages of this bacterium were detected in infertile women but still their role in infertility remain unclear. Among tested infertile women for *U. urealyticum*, 16.0% were positive. The results were lower than those by Fatlawy and Abduljabbar (2019) who found that 26.81% of infertile women from Baghdad, Iraq were positive, and in Iran by (Peerayeh et al., 2006) (Mortazavi et al., 2021) who found that 30.7% and 38.13% of the infertile women were positive for this pathogen respectively.

No *M. genitalium* was detected in the current study. This result disagreed with those of Fatlawy and Abdul Jabbar (2019) from Iraq, who found *M. genitalium* in 5.85% infertile women and Edberg (2010) found 7.7% and Grzeško et al (2009) from Poland 19.6%.

This difference between our results and other studies could be partially due to differences in the study population size, methods of diagnosis, sample collection and geographical distribution.

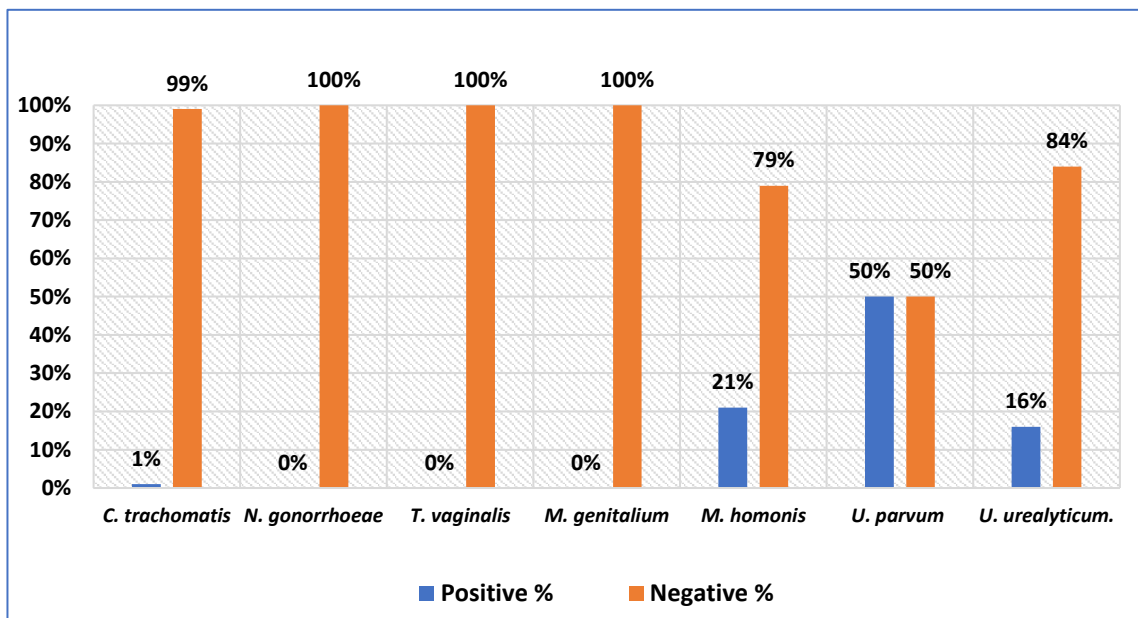


Fig. (1): Prevalence of STDs among infertile women using multiplex real-time PCR.

3.2 Distribution of the causative agents of STDs among different age groups.

In the present study, *C. trachomatis* was identified in age group 30–39 years, with a prevalence rate of 2.08%. However, no significant association ($p= 0.579$) was found between this pathogen and age groups as shown

in table (1), Similar results were found by Mawak et al (2011) in which 1.7% of *C. trachomatis* was detected among women with age group 14-39 years. A possible explanation could be our population's late onset of sexual activity.

A high percentage (70.59%) of *U. parvum* was detected in age group 40-49 and lower percentage (43.75%) in age group 30-39. Similar results were found by (De Francesco *et al.*, 2009) who did not find any significant association between *U. parvum* and studied age groups.

The highest percentage of *U. urealyticum* (20.0%) was found in age group 20-29 years and the lowest percentage (11.76%) in age group 40-49 year. However, no significant association was found between age groups and *U. urealyticum* ($p=0.699$). These results were similar to the results of Javadinia co-workers (2017) and Zdrodowska–Stefanow co-worker (2006) who found that the rate of infection with this pathogen was decreased as the age increased. The possible explanation for decreasing

infection with increasing of age is the decreasing of sexual activities as well as production of immunity.

The highest percentage (31.43%) of *M. hominis* was found in age group 20-29, while the lowest percentage (14.58%) was in group 30-39 but no significant association was detected between *M. hominis* and age groups ($p=0.165$). Similar results were reported by Majhi & Co-worker (2022) and Zdrodowska – Stefanow co-worker (2006) who also found the highest prevalence of *M. hominis* in the age group 20-24 and 26-30 years respectively. Infections with *M. hominis* were more frequent among patients with multiparity, multiple sexual partners, intrauterine contraceptive devices, lower socioeconomic status, and lower educational status (Majhi *et al.*, 2022).

Table (1): Distribution of the causative agents of STDs among age groups:

Variable	n=100	<i>C. trachomatis</i>			<i>M. hominis</i>			<i>U. parvum</i>			<i>U. urealyticum</i>			
		Patients	-ve	+ve	P value	-ve	+ve	P value	-ve	+ve	P value	-ve	+ve	P value
Age (Year)														
20-29 y	35	35	0	0.579	24	11	0.165	18	17	0.16	28	7	0.699	
30-39 y	48	47	1		41	7		27	21		41	7		
40-49 y	17	17	0		14	3		5	12		15	2		

p values were determined using the chi-square test. p values < 0.05 were considered statistically significant.

3.3 Distribution of the causative agents of STDs among different levels of education.

No significant association ($p=0.296$) was found between *C. trachomatis* infection and levels of education as shown in table (2). Our results were similar to those of Mabaso and co-workers (2022) who found that the level of education was not a significant factor in *C. trachomatis* infection. This may be because the rapid development of information technologies and the wide use of the internet have made comprehensive knowledge of STD prevention easily available through several media (Hu *et al.*, 2021).

In the current study, the education level was not statistically significant with *U. parvum* ($p=1.00$). current study is similar to the findings of Karim and co-workers (2020) who found similar percentages of *U. parvum* among literate and illiterate patients.

In our study, no significant association was found between *M. hominis* ($p=0.59$) and *U. urealyticum* ($p=0.861$) with the level of education as shown in table (2). The results agreed with those of Majhi & Co-worker (2022) who did not find significant association between these two pathogens and levels of education.

Table (2): Distribution of the causative agents of STDs according to the level of education.

Variable	n=100	<i>C. trachomatis</i>			<i>M. hominis</i>			<i>U. parvum</i>			<i>U. urealyticum</i>		
		Patients	-ve	+ve	P value	-ve	+ve	P value	-ve	+ve	P value	-ve	+ve
Educated	52	52	0	0.296	40	12	0.596	26	26	1:00	44	8	0.861
non-educated	48	47	1		39	9		24	24		40	8	

p values were determined using the chi-square test. p values < 0.05 were considered statistically significant.

3.4 Distribution of the causative agents of STDs according to the duration of infertility

The highest rate of *C. trachomatis* infection was 16.67% among patients with 11-15 years of infertility duration and a significant association ($p= 0.001$) was found between *C. trachomatis* infection and duration of infertility as shown in table (3). The results of the current study were similar to those of Mohammed and co-workers (2017) who found *C. trachomatis* in 13.75% among Iraqi infertile women. Although the highest rate (41.92%) of *U. parvum* detection was among infertility duration from 1-5 years but without significant association ($p= 0.231$). The presence of these organisms in the sterile part of the genital tract may induce long-lasting subacute inflammation that may be the cause of the upper genital tract disorders e.g., infertility (Urszula *et al.*, 2014).

A highest rate (47.61%) of *M. hominis* was detected in patients with duration of infertility

from 1 to 5 years but without significant association ($p= 0.393$). Similar results were found by Alagouri & Mohammad (2013).

A highest percentage (24.13%) of *U. urealyticum* was detected in women with duration of infertility from 6-10 years and the lowest percentage was 14.51% in those with duration of infertility from 1-5 years, while no infection with pathogen was detected in women with duration of infertility from 11-15 and 16-20 years. No significant association ($p= 0.355$) was found between *U. urealyticum* and duration of infertility. Similar results were found by Fenkci *et al* (2022) in Turkey who found *U. urealyticum* infection more commonly associated with young ages than older ages. The possible explanation for prevalence of *U. urealyticum* infection among young ages probably due to the role of men in the transmission of this pathogen.

Table (3): Distribution of the causative agents of STDs according to the duration of infertility

Variable	n=100	<i>C. trachomatis</i>			<i>M. hominis</i>			<i>U. parvum</i>			<i>U. urealyticum</i>		
		Patients	-ve	+ve	P value	-ve	+ve	P value	-ve	+ve	P value	-ve	+ve
1-5 y	62	62	0	*0.001	52	10	0.393	36	26	0.231	53	9	0.355
6-10 y	29	29	0		20	9		11	18		22	7	
11-15 y	6	5	1		5	1		2	4		6	0	
16-20 y	3	3	0		2	1		1	2		3	0	

p values were determined using the chi-square test. p values < 0.05 were considered statistically significant.

4. CONCLUSION

Based on the results of the current study that both *N. gonorrhoeae* and *C. trachomatis* are very low in the birth canal of infertile women, while other sexually transmitted pathogens like *U. parvum*, *M. hominis* and *U. urealyticum* found more commonly which demands more studies to elucidate their association and role in the pathogenesis of infertility.

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پوخته

نهخوشییه سینکسییهکان له ریگهی بهرکهوتنی سینکسیشهوه دهگوازینیهوه ههروهه به نهخوشییهکی سینکسی یان نهخوشی سینکسی ناودهبریت، و کیشیهکی تهنروستی گهوره دروست دهکهن بهتاییهتی له ولاتانی تازهپینگهیشتودا. نامانجی سهههکی نهه تویرینهوهیهی نیستا دیاریکردنی هۆکاره باوهکانی بهکتريا و پرتوتوزونالی ههوکردنی سینکسییه له نیوان ژناتی نهزوکدا به بهکارهینانی تهکنیکهکانی PCR له کاتی راستهقینهدا له پاریزگای دۆهوک له عیراق. نهه تویرینهوهیه بربرهیی لهسهه 100 ژنه نهزوک له شوپاتی 2022 تا شوپاتی 2023 نهجامدراوه. پرسیارنامهی پیکهاتهدار بۆ پیوانهکردنی دیموگرافی، هۆکارهکانی مهترسی و تاییهتمهندییه کلینیکیهکان داریژراوه. پشکنینی ژن له ههه کهسینک کۆکرایهوه و کارلنیکي زنجیرهی پۆلیمیراز له کاتی راستهقینهدا بهکارهینرا بۆ دیاریکردنی *M. genitalium*، *N. gonorrhoeae*، *T. vaginalis*، *C. trachomatis* و *U. parvum*.

مامناوهندی تههمنی بهشداربووهکه 32.97 سأل بوو. (SD 6.90 ±) له کۆی 100 ژنه نهزوکهکه، 68 (68%) یان پۆزهتیف بوون بۆ یهکتیک یان زیاتر له هۆکارهکانی هۆکار. بهرترین ریژهی تووشبوون (50.0%) بههۆی *U. gonorrhoea*، *T. vaginalis* و *M. genitalium* بووه. نیمه ریژهی تووشبوونمان زیاتر له نیوان خههکی ناوچه شاریهکان دۆزیهوه (65.00%)، بهلام له رووی نامارییهوه گرنگ نهبوو. زۆرجار نیشانه کلینیکیهکان پهیههندییان به ههوکردنی نهخوشییه سینکسییهکانهوه ههیه و گرنگن.

تویرینهوهی نیستا دهرخستوه، وهه زۆریهی تویرینهوهکان، که نهخوشییه سینکسییهکان نهخشیکي جیناتی بالادهستی نۆتۆسۆمییه، و بلابوونهوهی نهخوشییه سینکسییهکان لهنیو ژناتی نهزوکدا زیاتر بووه.

الخلاصة

يُشار أيضًا إلى العدوى المنقولة جنسيًا التي تنتقل عن طريق الاتصال الجنسي على أنها مرض ينتقل عن طريق الاتصال الجنسي أو مرض تناسلي، ويتم نقلها أيضًا من خلال الاتصال الجنسي وتشكل مشكلة صحية كبيرة خاصة في البلدان النامية. الهدف الرئيسي من الدراسة الحالية هو الكشف عن الأسباب البكتيرية والأولية الشائعة للعدوى المنقولة جنسيًا بين النساء المصابات بالعمم باستخدام تقنية تفاعل البوليميراز المتسلسل في الوقت الحقيقي في محافظة دهوك، العراق. تم إجراء هذه الدراسة المقطعية على 100 امرأة مصابة بالعمم من فبراير 2022 حتى فبراير 2023. تم تصميم الاستبيان المنظم والمنظم لقياس العوامل الديموغرافية وعوامل الخطر والخصائص السريرية. جمعت المسحات المهبلية من كل شخص واستخدمت في الوقت الحقيقي لتفاعل تفاعل البوليميراز المتسلسل للكشف عن *M. genitalium* و *U. urealyticum* و *C. trachomatis* و *T. vaginalis* و *N. gonorrhoeae*.

كان متوسط عمر المشارك 32.97 سنة. (SD 6.90 ±) من بين 100 امرأة مصابة بالعمم، كانت 68 (68%) موجبة لواحد أو أكثر من العوامل المسببة. أعلى نسبة إصابة (50.0%) سببها *U. urealyticum*، *T. vaginalis* و *M. genitalium*. وجدنا معدل إصابة أعلى بين الأشخاص من المناطق الحضرية (65.00%)، ولكن ليس مهمًا من الناحية الإحصائية. غالبًا ما ترتبط الأعراض السريرية بعدوى الأمراض المنقولة بالاتصال الجنسي وهي مهمة.

أظهرت الدراسة الحالية، أن الأمراض المنقولة بالاتصال الجنسي هي نمط وراثي سائد، وكان انتشار العدوى المنقولة جنسيًا أعلى بين النساء المصابات بالعمم.