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Molecular Detection of Some Sexually Transmitted Bacteria and *Trichomonas vaginalis* in Iraqi Married Couples

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Abstract: Sexually transmitted infections (STIs) are a very frequent and under-diagnosed cause of illness worldwide. Sexually transmitted infections can adversely affect a woman's pregnancy and the health of the developing fetus. This study aimed to investigate the prevalence rate of STI pathogens including *Chlamydia trachomatis*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* in symptomatic Iraqi patients of married couples by molecular method. A total of 108 clinical samples were collected from symptomatic patients of married couples which included (cervical swabs from women and Semen from men) who were referred to two specialized hospitals and one private specialized hospital in Baghdad, Iraq, between November 2020 to march 2021, also 50 healthy controls were contributed in this study. After DNA extraction of samples, the PCR was carried out with specific primers. Finally, the results were analyzed. Among 108 symptomatic patients, the prevalence of *Chlamydia trachomatis*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* was 16 (14.8%), 11 (10.2%), 8 (7.4%), and 6 (5.5%), respectively. The frequency of the bacterial infections (32.4%) was more than the infection with *T. vaginalis* (5.5%). According to the age group it was found that the bacterial and parasitic infections were high in the patients with the age more than 26 years old in comparison with the younger patients and the most prevalent microorganism was *Chlamydia trachomatis* among all age groups. Tow infections (4%) were recorded among healthy individuals by *M. hominis*, and *T. vaginalis*. It was concluded polymerase chain reaction is a good diagnostic tool for sexually transmitted infections because it has high sensitivity and specificity, and it was found that *C. trachomatis*, and *Mycoplasma hominis* are the most prevalent among symptomatic patients.

Keywords: Sexually transmitted bacteria, *Trichomonas vaginalis*, PCR

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Introduction

Sexually transmitted infections (STIs) cause substantial morbidity and economic cost worldwide. *Chlamydia trachomatis* (CT) infection and *Neisseria gonorrhoeae* (NG) infection are among the most common bacterial STIs (1). Globally, 374 million new infections of the four curable STIs occur annually, these infections have significant public health consequences including infertility, ectopic pregnancies, prematurity and neonatal deaths, as well as increased risk of HIV acquisition (2). Among the married couples, many of these infections are asymptomatic for pregnant women, maternal infection with *Chlamydia trachomatis, Neisseria gonorrhoeae*, and *Trichomonas vaginalis* have all been associated with premature rupture of membranes, preterm birth, and low birth weight (3). Maternal infection with *N. gonorrhea* and *C. trachomatis* at the time of vaginal delivery can result in directly infecting the infant's eyes

and respiratory tract, causing neonatal conjunctivitis and pneumonia (4). WHO recommends quality-assured molecular assays for treatment of people with symptoms of NG, CT and *Trichomonas vaginalis* (TV), If quality-assured molecular assays are not available, treatment-based syndromic approach is recommended (5).

The high prevalence of STIs in the middle-east region, combined with an increased antibiotic resistance of several bacterial strains, there was necessitate the generation of basic epidemiological data to assess the burden of STD in Iraq. The molecular diagnostic assays are necessary to understand the impact of this interplay and provide insights into the STD profile and trends in Iraq. In our study, we aim to determine the prevalence of STD bacterial pathogens using the conventional PCR technique, we will analyze data in correspondence to age and gender, and we will compare our results to international studies.

Materials and methods

DNA extraction and identification of specific genes for detection the sexually transmitted bacterial and *T. vaginalis* by PCR

Bacterial DNA was extracted from all 108 clinical samples (54 of cervical swabs from women and 54 of Semen from men) using ready kits (Promega, USA). Purity of the isolated DNA was monitored by NanoDropper 2000 (Thermo Scientific, USA). The PCR reactions for detection *16S rRNA, TVK3-TVK7, dcmH,* and *ofr8* genes for molecular identification of the microorganisms, *Mycoplasma hominis*, *Trichomonas vaginalis, Neisseria gonorrhoeae* and *Chlamydia trachomatis* respectively were done within a total volume of 20 µL. The mixture of reaction contained Master Mix (10 µL), 1 µL of forward and reverse primers, DNA template 3 µL and Nuclease Free Water 5 µL. The Primer sequences, which were used for detection of genes of the sexually transmitted bacterial and *T. vaginalis* in this study, were as in Table (1).

Table (1): Conditions of PCR reaction for detection the sexually transmitted bacterial and *T. vaginalis* infections in a sample of Iraqi couples patients

The bacterial species	Target Gene	Primer name	Oligonucleotide primer Sequence(5-3)	Ambilicon size(bp)	The Reference
M. hominis	*16S rRNA*	RNA H1, F	CAATGGCTAATGCCGGATAC	335	Ataee *et al*., (6)
		RNA H2, F	GGTACCGTCAGTCTGCAAT		
T. vaginalis	TVK3-TVK7	TV3, F	ATTGTCGAACATTGGTCTTACCCTC	300	Lawing *et al*., (7)
		TV7, R	TCTGTGCCGTCTTCAAGTATGC		
N. gonorrhoeae	*dcmH*	F	GCCTCGCGGCTTGGCTA	496	Karimpour *et al.,* (8)
		R	GGCGCAGACGGTTACTTAAGCAGGA		
C. trachomatis	*orf 8*	F	CTAGGCGTTTGTACTCCGTCA	200	Dahaghin *et al*., (9)
		R	TCCTCAGGAGTTTATGCACT		

For amplification of genes, PCR conditions were carried out by the thermocycler (Applied Biosystems, Malaysia) according to the conditions of the previous studies with the modifications as mentioned in table 2. Agarose gel electrophoresis was done a 1.2 % agarose gel at 80V for 2 hours. After electrophoresis fragments were stained by ethidium bromide, and then visualized with ultraviolet light.

Table (2): Conditions of PCR reaction for detection the sexually transmitted bacterial and *T. vaginalis* infections in a sample of Iraqi couples patients

Bacterial species	Target gene	PCR conditions	No. of cycles	Reference
M. hominis	*16S rRNA*	Initial denaturation at 95°C for 4 min 95℃ for 1 min 56 ℃ for 30 sec 72 ℃ for 1min final extension at 72 ℃ for 5 min	1 cycle 35 cycle 1 cycle	This study
T. vaginalis	TVK3-TVK7	Initial denaturation at 94° C for 5 min 94℃ for 1 min 60 ℃ for 30 sec 72°C for 1 min final extension at 72 °C for 5 min	1 cycle 30 cycle 1 cycle	This study
N. gonorrhoeae	*dcmH*	Initial denaturation at 95°C for 5 min 95℃ for 30 sec 57 ℃ for 1 min 70 ℃ for 30 sec final extension at 70 ℃ for 5 min	1 cycle 30 cycle 1 cycle	This study
C. trachomatis	*orf 8*	Initial denaturation at 95°C for 5 min 95℃ for 30 sec 57 ℃ for 1 min 70 ℃ for 30 sec final extension at 70 ℃ for 5 min	1 cycle 30 cycle 1 cycle	This study

Results and discussion

Detection of the sexually transmitted bacterial and *T. vaginalis* genes by Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from all clinical samples of infected patients and healthy control. Extraction genomic DNA from all 108 infected patients and 50 of healthy individuals that was confirmed as bands by gel electrophoresis. DNA concentration and purity were measured by Nanodrop spectrophotometer, all the isolates had DNA concentration between (50-100 ng/µl) and purity of the DNA were (1.4- 2) (Figure1).

Figure (1): Agarose gel electrophoresis of extracted DNA to check purity and integrity. Lane 1-10: DNA of different clinical samples, Lane NC: Negative control. (70 V/ 30 min.)

In order to detect the presence of species-specific genes (*16S rRNA, TVK3-TVK7, dcmH,* and *ofr8* genes) and the determination of the prevalence of each gene among the clinical samples, polymerase chain reaction (PCR) for each DNA extracted sample have been used. The PCR products have been confirmed by the analysis of the bands on gel electrophoresis and by comparing their molecular weight with 100 bp DNA Ladder.

Each DNA extracted sample was subjected to PCR reaction with primer sets of *16S rRNA* (335 bp), *TVK3-TVK7* (300bp), *dcmH* (694bp), and *ofr8* (200bp). The results of detection of these genes by PCR in all isolates were shown in figures 2, 3 and 4, and the distribution of these genes among clinical bacterial isolates and *T. vaginalis*, also the prevalence of theses microorganisms among clinical samples and healthy control were demonstrated in the Tables (4) and (5).

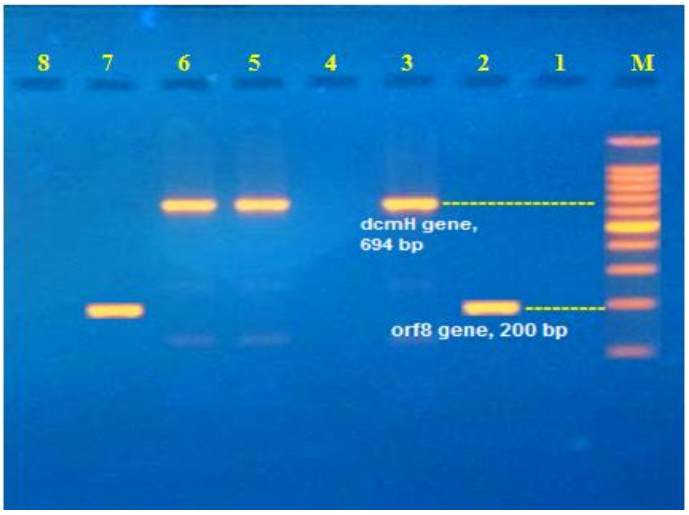

Figure (2): Agarose gel electrophoresis of PCR products for the detection genes *dcmH* (694bp) and orf8 (200bp). Lane M: 100bp DNA ladder; lanes 1-8: *positive results of N. gonorhoeae* isolates (lanes 3, 5 and 6), positive results of *C. trachomatis* isolates (lanes 2 and 7); lane 1: negative control. (70V for 2hrs).

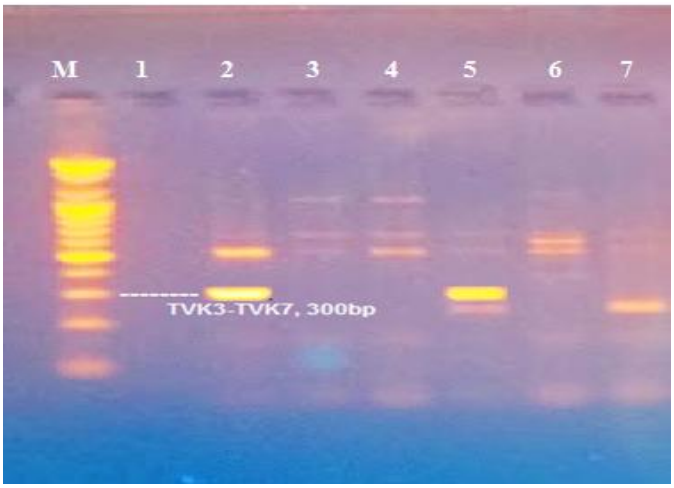

Figure (3): Agarose gel electrophoresis of PCR products for *T. vaginalis* detection gene (TVK3-TVK7), Positive results (lanes 2 and 5); Lane M: 100bp DNA ladder;lane 1: negative control. (70V for 2hrs)

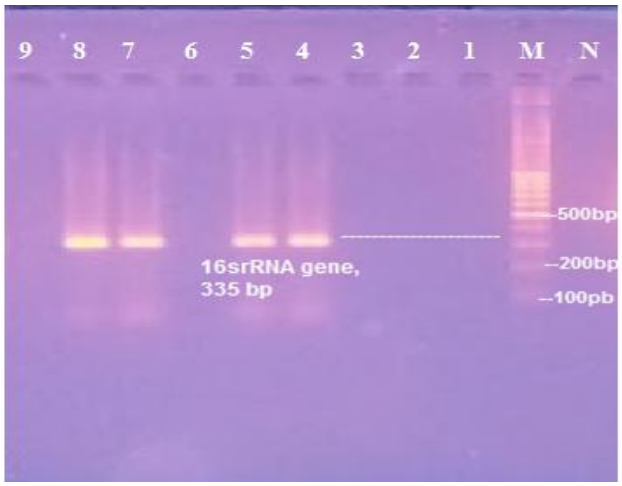

Figure (4): Agarose gel electrophoresis of PCR products for *M. hominis* detection gene (16S rRNA), Positive results (lanes 4, 5, 7 and 8); Lane M: 100bp DNA ladder;lane 1: negative control. (70V for 2hrs).

The results of PCR amplifications (Figures 2 to 4 and Tables 4 and 5) revealed that these genes exhibited high specificity for identification of sexually transmitted infectious agents, where, among 107 of symptomatic patients, the prevalence of *Chlamydia trachomatis*, *Mycoplasma hominis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* was 16 (14.8%), 11 (10.2%), 8 (7.4%), and 6 (5.5%), respectively. The frequency of the bacterial infections (32.4%) was more than the infection with *T. vaginalis* (5.5%). According to the age group it was found that the bacterial and parasitic infections was high in the patients with the age more than 26 years old in comparison with the younger patients and the most prevalent microorganism was *Chlamydia trachomatis* among all age groups. Tow infections (4%) were recorded among healthy individuals by *M. hominis*, and *T. vaginalis*.

Table (4): The distribution bacterial and *T. vaginalis* infections among different age groups of infected patients and healthy individuals

Age (years)	Infection percentage (%)	Bacterial frequency (%)	Parasite frequency (%)
18-25	25.9% (28/108)	35.7 (10/28)	7.1 (2/28)
26-35	36.1% (39/108)	33.3 (13/39)	0
36-45	37.9% (41/108)	29.3 (12/41)	9.8 (4/41)
Healthy control	4% (2/50)	2% (1/50)	2% (1/50)

Healthy control (18-45 years)

Table (5): The distribution of the sexually transmitted bacteria and *T. vaginalis* among different age groups of infected patients and healthy individuals

Age (years)	No. of couples samples	Pathogenic bacteria			Parasite
		C. trichomatis	*M. hominis*	*N. gonorrhorea*	*T. vaginalis*
18-25	28	4	5	1	2
26-35	39	8	2	3	0
36-45	41	4	4	4	4
Total	108	16	11	8	6
		14.8% (16/108)	10.2% (11/108)	7.4% (8/108)	5.5% (6/108)
Healthy control	50	0	2% (1/50)	0	2% (1/50)

Healthy control (18-45 years)

Sexually transmitted infections (STIs) and reproductive tract infections (RTIs) are a significant cause of global burden of disease. Of the eight pathogens of highest public health importance, four are curable, *Treponema pallidum*, *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) and *Trichomonas vaginalis* (TV) while the other four of viral aetiology are not, hepatitis B, herpes simplex virus, HIV and human papillomavirus (10).

The study of Kriesel *et al*. (11), demonstrated that *C. trachomatis* were the most prevalent among the infected patients, this study used multiple PCR for detection nine of sexually transmitted infectious agents, two hundred and ninety-five clinical specimens (Urine, urethral/cervical swabs, oral swabs, rectal swabs, and ulcer swabs) were tested. Among the tested samples, it was found *C. trachomatis* in 39 (13%), *N. gonorrhoeae* in 20 (7%), *T. vaginalis* in nine (3%), multiplex PCR STI testing has the potential to improve public health by providing rapid, sensitive, and reliable results within the clinic or nearby laboratory.

The prevalence of *C. trachomatis* and *N. gonorrhoeae* in pregnant women ranged between 1.0%-36.8% and 0-14.2% worldwide, respectively. The most common diagnostic method is the Nucleic acid amplification test (NAAT). In pregnancy, chlamydia is associated with preterm birth, spontaneous miscarriage, stillbirth and neonatal conjunctivitis, while gonorrhoea is mainly associated with preterm birth and stillbirth(12). One of the previous studies included out of the 400 women, 11 percent carried *Mycoplasma hominis*, 44.75 percent *Ureaplasma urealyticum* and 7.75 percent *Chlamydia trachomatis*. Positivity was more frequent among those having several partners and those not using condoms regularly. The author thinks the pathogen infection rate found can be one of the main causes of urogenital inflammations, fertility problems and premature deliveries (13).

The incidence of *Ureaplasma urealyticum* and *Mycoplasma hominis* strains cultured from the genital discharges of sexually active individuals in Hungary, demonstrated that *U. urealyticum* was isolated in 12.54 % in the cervix and 4.1 % in the male urethra, while *M. hominis* was isolated in 1.33 % in the cervix and 0.51 % in the male urethra. The affected age group was between 21 and 60 years old (14). The previous findings of Miller *et al*. (15) were not agreed with our

results, where it revealed that the prevalence of Chlamydia and/or gonorrhoea ranged from 23.0% among 15–19-year-olds to 3.5% among those 40 years and older. In the adjusted analysis younger age, female sex, lower socioeconomic status, the use of alcohol and tobacco, and the structure of community health services were independently associated with a higher prevalence of bacterial STI. The comprehensive epidemiological research of *C. trachomatis* infection in the Middle East and north Africa indicated that *C. trachomatis* infection prevalence in the population at large in the Middle East and north Africa is at 3%, similar to other regions, but higher than expected given these countries' sexually conservative norms. The high prevalence (>10%) in infertility clinic attendees and in women with miscarriage, provides suggestive evidence for the potential role of *C. trachomatis* in poor reproductive outcomes in the Middle East and north Africa (16).

This study indicated to the presence of *T. vaginalis* among married couples even though in low frequency (5.5%) but may be led to the additional risk factor and more complications. *Trichomonas vaginalis* infections in men are traditionally considered to be benign and consequently have been overlooked. However, men with this common sexually transmitted infection can experience urethritis, prostatitis, reduced fertility, and amplified human immunodeficiency virus risk(17). The importance of *T. vaginalis* infection squeal in women, including increased risk of human immunodeficiency virus (HIV) acquisition, cervical cancer, preterm birth, and other adverse pregnancy outcomes. Many diagnostic methods, including point-of-care assays and multiple nucleic acid amplification tests, can be performed on a variety of genital specimens in women and men, including urine, allowing more accurate and convenient testing and screening of those at risk for infection (18). Chlamydia, trichomoniasis, and genital herpes showed a trend of increasing incidence rates from 2010 to 2019. chlamydia tended to be older in southern sub-Saharan Africa (25-29 years vs. 30-34 years) but younger in Australasia (40-44 years vs. 25-29 years (19). A cross-sectional study estimating the prevalence of *T. vaginalis* infection in the Turkish general population found that the mean prevalence of infection was found to be 5.94%, 2.87% in men, and 6.17% in women. *T. vaginalis* is still an important health problem among the Turkish population. The prevalence varies depending on the socioeconomic structure of the region, the lifestyle of the person, the method used in the study, the size of the population, and the clinical condition of patients (20,21).

Conclusion

According to our results, the PCR method, especially detecting the species-specific genes, was more sensitive than the direct examination and conventional culture medium methods. it was found that *C. trachomatis*, and *Mycoplasma hominis* are the most prevalent among symptomatic patients of the married couples.

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