Original Article

Prevalence and Molecular detection of bacteria isolated from sexually transmitted disease in Al-Anbar Province

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ABSTRACT

This study is the first in the Al-Anbar governorate with this design aim to the detection of Sexually transmitted diseases (STDs) by using RT-PCR. A total of (70) samples were obtained from women18 years of age and over, with or without symptoms of genital tract infection, randomly collected and carried out intermittently in Al-Anbar province between march 2020 and January 2021. STIs bacteria were detected by Real-time PCR. Out of 70 samples with or without symptoms of genital tract infection for STDs, bacteria detection using real-time PCR technique. The results were 36 (51%) and 34 (48.5%), negative and positive, respectively. The results revealed four genera of sexually transmitted bacteria causative agents of sexually transmitted diseases. These included bacteria from *Ureaplasma parvum* that accounted for the highest percentage of infections with sexually transmitted diseases, with (20) 28% of 34 (48.5%) positive results. Although *Mycoplasma genitalium,* was the lowest at (2) 2.8%, detecting two other bacteria including *Chlamydia trachomatis,* was identified with a rate of (6) 8.5%, and *Mycoplasma hominis* bacteria was identified with the same percentage. The results supported the need for greater STI prevention knowledge. Seven STI pathogens were successfully identified in our research population using the real-time PCR approach. The *Mycoplasma genitalium* pathogen had the lowest detection rates compared to the typically high *Ureaplasma parvum* pathogen detection rates.

GRAPHICAL ABSTRACT
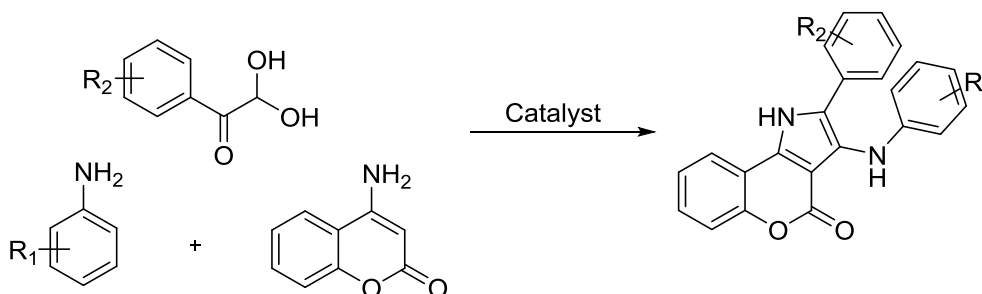
Introduction

Infections known as sexually transmitted diseases (STDs), frequently communicated through sexual activity and are an epidemiologically significant transmission mechanism, can also disseminate through unsterilized needles. More than 30 distinct STIs exist, are brought on by several pathogens such as bacteria, parasites, and viruses. Chlamydia, gonorrhea, and syphilis are bacterial STIs. Genital herpes, HIV/AIDS, and genital warts are examples of viral STIs. Trichomoniasis is one of the parasitic STIs. Non-sexual contact with donor tissue, blood, or other bodily fluids can transfer some STIs, nursing, or delivery in addition to the conventional method of sexual transmission. In the industrialized world, STI diagnostic tests are often readily available but often lacking in the developing world. This poses a serious public health issue for women and men worldwide [1].

According to WHO reports (2012) [2] there are 340 million cases of trichomoniasis syphilis, gonorrhea, and chlamydia in the 15-49 age group. HPV infections constitute the most common, estimated at 5,000,000 cases/year, and the proportion of developing countries is 80% [2, 3]. Most sexually transmitted diseases are without symptoms and, therefore, difficult to diagnose and may not be diagnosed.

Infection with sexually transmitted diseases sharing risks of contracting human immunodeficiency virus (HIV), as the epidemiology indicates that infection is mainly among men [4].

The World Health Organization recorded a record number of HIV infections during the past seven years, with an infection rate of about 3,294 per year. The world has begun to reduce the rate of HIV infection by preventing sexually transmitted diseases [5].

In practically every country, the frequency of STDs has recently increased, particularly among young people. As a result, these disorders are presently receiving considerable attention from the medical community [6]. More than 500 million people worldwide have herpes simplex type 2 each year, and many other viral sexually transmitted infections are also common. In addition, human papillomavirus (HPV) infection, which affects more than 290 million women globally, which affects both men and women, is the most typical STI.

Infection with transmitted diseases is not limited to men but also includes women. These diseases can be transmitted to their children. The most important of these diseases is congenital syphilis, where maternal non-treponemal and treponemal IgG antibodies are transmitted from mother to child [7].

Gonorrhea is the second most common sexually transmitted bacterial infection worldwide, caused by *Neisseria gonorrhoea* [8].

Bacteria can also ascend to the upper genital tract from the site of infection to cause pelvic infection and bacteremia; therefore, early diagnosis and appropriate treatment will prevent serious disease complications because increasing resistance to treatment for gonorrhea may seriously affect the infected [9].

Furthermore, there are non-viral sexually transmitted diseases, as *Trichomonas vaginalis* is considered one of the most common non-viral sexually transmitted diseases and the most prevalent in the world, as these bacteria are associated with poor reproductive health, premature births, and low birth weight of the child [10].

About 357 million new instances of four STIs, including 5.6 million each of syphilis, 78 million Neisseria gonorrhoeae, 143 million trichomoniasis, and 131 million Chlamydia trachomatis, have been reported among people aged 15 to 49 globally [11]. HPV is the most common bacterial infection transmitted sexually in the United States and is frequently spread by asymptomatic people [12]. Gonorrhea infection rates have increased to 79 percent since 2008, especially among men. The second most reported STI in Europe in 2016 was gonorrhea, with over 75.000 cases recorded; in Spain, 6.456 instances were reported, with 83% of the patients being male. Gonorrhea infection rates have increased to 79 percent since 2008, particularly among men. The second-most often reported STI in Europe in 2016 was gonorrhea, with over 75.000 cases recorded; in Spain, 6.456 instances were

reported, with 83% of cases being male. Gonorrhea infection rates have increased to 79 percent since 2008, particularly among men. The second-most reported STI in Europe in 2016 was gonorrhea, with over 75.000 cases recorded; in Spain, 6.456 cases were reported, with 83 percent being male [13].

Since many sexually transmitted illnesses are subclinical or asymptomatic, manual techniques are seldom used to diagnose them, making them a hidden pandemic that increases the risk of spreading the disease to others. Endometritis is one of the symptoms and indicators of STIs, along with vaginal discharge, penile discharge, ulcers on or around the genitalia, itching, trouble peeing, and pelvic discomfort. STIs can be passed from a mother to a baby before or during delivery, which might harm the baby's health. Most people do not even realize they have Ureaplasma in their bodies. The male and female reproductive systems have been related to Ureaplasma, which is also transferred from the mother to the infant. It was shown that Ureaplasma vaginal infections were more common after several sexual partners. Some STIs have been linked to both male and female infertility. Although M. genitalium has been linked to clinical cervicitis, PID, premature birth, recurrent abortion, and infertility, the most common symptoms of gonorrhea in both men and women are urethritis and cervicitis, respectively. Men's and women's pelvic inflammatory disease and epididymitis are the most prevalent local consequences of gonorrhea. However, appropriate screening may prevent many STDs, especially in high-risk individuals. These diagnostic assays enable high-risk group screening, epidemic investigations, the discovery of antibiotic resistance, and treatment monitoring [14]. The need for new technologies, including nucleic acid amplification platforms like multiplex PCR assays and real-time PCR for disease detection, has recently been recognized as a means of combating this illness [15, 16]. The study's main objectives were to determine the point prevalence of STIs and the molecular identification of bacteria isolated from STIs in the Al-Anbar governorate.

Materials and Methods

The experiment design

Between March 2020 and January 2021, the Al-Anbar administration performed this study sporadically. Seventy women who requested gynecological exams in private clinics provided samples. In Anbar, these clinics treat a sizable number of patients from various socioeconomic backgrounds. Women between 18 and 55, with or without signs of genital tract infection, were included in that study. The questionnaire forms created for the study contained general information about the willing participant. A dermatologist and gynecologist then performed a clinical examination and collected samples, utilizing laboratory testing and STD screening.

Extraction of DNA

Seventy vaginal samples of DNA were extracted in Spain by the manufacturer's instructions using a kit called the VIASURE RNA-DNA Extraction Kit. The extraction kit was kept at room temperature (RT) (15–30 °C) until the day of the experiment since any dried or thawed material (such as carrier RNA, proteinase K) should be stored at -20 °C and wash buffer at RT. Samples were kept after being frozen in a clean Eppendorf basin. Following the preparation of our samples, kits, and equipment, there were preliminary procedures before RNA isolation by the Spain (manufacturer) methodology (VIASURE RNA-DNA Extraction Kit).

Real-time PCR for the detection of ten pathogens

Real-time PCR detection of sexually transmitted diseases (STDs) by the VIASURE Company's Spin DNA & RNA RT-PCR kit technique.).

Statistical analysis

To determine how various factors affected the study %, the Statistical Analysis System- SAS (2012) tool was employed. The Chi-square test was used to compare percentages significantly [17].

Results and discussion

Description of the isolated samples

An estimated one million illnesses are discovered per day, and sexually transmitted diseases are a serious global health issue. These infections can range from mild to severe and increase the risk of contracting HIV. In addition to the huge amounts of disease and death caused by contracting these diseases, these diseases place a heavy economic burden on society [18].

An evaluation of STD detection techniques was performed as part of the study. To make a significant contribution to using the quickest and most precise approach for early identification of STDs and providing the required care.

RT-PCR results

Many complications of sexually transmitted diseases can be prevented by appropriately screening individuals at high risk of contracting these diseases by performing diagnostic tests for sexually transmitted diseases, which help in diagnosing atypical cases, multiple infections, and infections without symptoms. But the test methods must be characterized by accuracy, capacity, accessibility, privacy, efficiency, sensitivity, reliability, ease of handling, and being at an appropriate cost [19].

For numerous reasons, including the recent identification of STDs as a serious public health concern, there is a lack of knowledge regarding the epidemiology of STDs in Iraq. This extensive clinical study provides crucial information about STIs' prevalence, pattern, and syndromic treatment.

In this research, the real-time PCR technique was used to accurately diagnose seven bacterial genera that cause sexually transmitted diseases, including Ureaplasma parvum, Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma urealyticum and Mycoplasma ho minis [20], The most effective way to find chlymedia infection, trachomatis infection is the culture technique. However, the newest approach has taken its place. The nucleic acid amplification test, of which there are several, is not culture. These tests have strong specificity (85%) and sensitivity (85%). (94 to 99.5 percent). when urethral cultures are matched with the endocervical and urethral samples.

This study included 70 samples of women aged 18 years or older who suffered vaginal discharge, abnormal vaginal bleeding, ulcers on or around the genitals, lower abdominal pain, and pelvic pain. The results were 36 (51%) and 34 (48.5%), negative and positive, respectively (Table 1).

The findings indicated that real-time PCR was utilized to diagnose sexually transmitted illnesses accurately and that there were four genera of sexually transmitted bacteria were the causal agents of disease. These included Ureaplasma parvum bacteria, which were responsible for most STD infections.

These included *Ureaplasma parvum* bacteria, which accounted for the highest percentage of infections with sexually transmitted diseases, as it was (20) 28% of 34 (48.5%) positive results with a significant value under $p < 0.01$.

U. parvum is closely related to the symptoms of sexually transmitted diseases in females, as it has a role in the inflammation of the female genitourinary system. In many cases, it causes cervicitis and bacterial vaginosis [21].

In addition, *Chlamydia trachomatis* was identified with a rate of (6) 8.5%, with the same percentage identified the *Mycoplasma hominis* bacteria, while *Mycoplasma genitalium* was the lowest at (2) 2.8% (Table 1).

The research results agreed with [22], who found that 35% of *Ureaplasma parvum* bacteria and 0.2% of the bacteria *Mycoplasma genitalium* caused sexually transmitted diseases.

This research showed a small prevalence of Chlamydia trachomatis (8.5%) and *Mycoplasma genitalium* (2.8%). However, the disappearance of other species of bacteria, such *as Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Ureaplasma urealyticum*

Compared to other research conducted in the area of the Middle East and North Africa, our findings [23, 24] indicated a frequency of 0.1 to 0.6% for N. gonorrhoea and 2.5 to 4.2% for C. trachomatis; however, the incidence of these bacteria is said to be higher in the UK, the United States, and France [25-27].

Table 1: Bacterial species isolated from STI and their percentage

Bacterial species	No. of negative samples	Percentage (%)	No. of positive samples	Percentage (%)	Chi-Square ($\chi2$┐┐┐┐)
Ureaplasma parvum	50	71%	20	28%	11.47 **
Neisseria gonorrhoeae	70	100%	0	0%	16.00 **
Chlamydia trachomatis	64	91%	6	8.5%	14.05 **
Mycoplasma genitalium	68	97%	2	2.8%	14.96 **
Trichomonas vaginalis	70	100%	0	0%	16.00 **
Ureaplasma urealyticum	70	100%	0	0%	16.00 **
Mycoplasma hominis	64	91%	6	8.5%	14.05 **
Chi-Square ($\chi2$)	-	8.923 **	-	8.923 **	-
** ($P\leq0.01$)					

The researchers discovered that opportunistic bacteria of the genus Mycoplasma, including both species (*Mycoplasma hominins* and *Mycoplasma genitalium*), as well as Ureaplasma due to its capacity to break down urea, are responsible for sexually transmitted diseases. Two kinds of Ureaplasma have been identified by researchers (U. parvum and U. urealyticum) More than 40% of individuals with inflammatory disorders of the urogenital system have mycoplasma and Ureaplasma; three species have the most therapeutic importance (M. genitalium, and M. hominis). Both men and women can develop urethritis due to Ureaplasma and mycoplasma, which may lead to cervicitis, cystitis, pregnancy difficulties in the post-partum period, and post-partum issues [28].

Researchers (2022) found that many symptoms appear in people with sexually transmitted diseases, and these symptoms included itching around the genital area 10.2%, abnormal vaginal discharge 12.7%, lower abdominal pain 19.5%, painful micturition 11.0% and genital ulcer 5.9%, as well as the prevalence of many syndromes as a result of sexually transmitted diseases, including genital ulcers 5.9%, enlarged inguinal lymph nodes 11.9%, pain in the lower abdomen 19.5%, and vaginal discharge syndrome 51.7% [29]

While the search results disagree with [30] because he found that *Mycoplasma genitalium* was the most commonly detectable pathogen in 67.5% of all women and concluded that it is possible to be double and triple infections with more than one type of bacteria which in agreement with the current study.

The results showed that some patients were infected with more than one species of bacteria that cause sexually transmitted diseases (Table 2, Figure 1 and 2).

In samples No. 5 and No.28, there are patients infected with three species that cause sexually transmitted diseases including, *Ureaplasma parvum*, *Chlamydia trachomatis,* and *Mycoplasma genitalium* while, samples No.10 and No. 12 are infected with *Ureaplasma parvum*, *Chlamydia trachomatis* and *Mycoplasma hominis*. Also, there are patients with two species of bacteria at the same time, including samples No. 8, No. 32, and No. 34 that are infected with *Ureaplasma parvum* and *Mycoplasma hominis*, and patients No. 19 and No. 31 that are infected with *Ureaplasma parvum* and *Chlamydia trachomatis* (Table 2, Figure 1 and 2).

The bacteria M. genitalium is associated with many harmful reproductive diseases and affects both sexes, as it affects women with endometritis, cervicitis, spontaneous abortion, and preterm birth, as well as urethritis in men [31, 32].

Studies have shown that Bacterium M. genitalium promotes HIV infection; studies have also shown that Mycoplasma hominis, and Ureaplasma urealyticum infect the genitourinary system of healthy patients [33].

Many studies are concerned with the mechanisms of resistance developed by bacteria. Understanding these mechanisms of antimicrobial action and the emergence of resistance is necessary to modify existing drugs and develop new ones directed against resistant bacteria [34].

Table 2: Bacterial species isolated from STI compared with the positive control

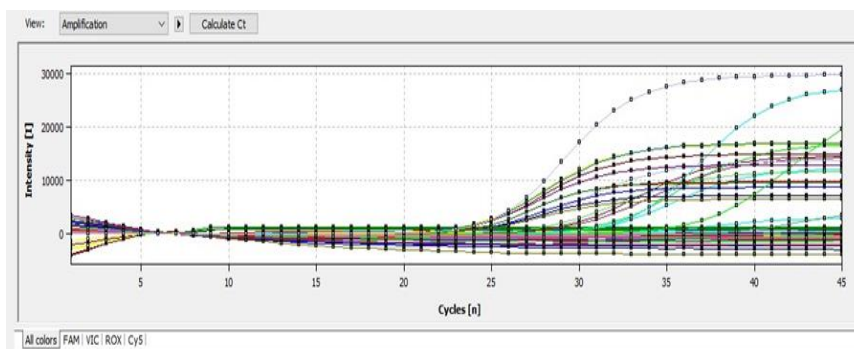| No.	Ureaplasma parvum	Neisseria gonorrhoeae	Chlamydia trachomatis	Mycoplasma genitalium	Trichomonas vaginalis	Ureaplasma urealyticum	Mycoplasma hominis
2	+	-	-	-	-	-	-
5	**+**	**-**	**+**	**+**	**-**	**-**	**-**
7	+	-	-	-	-	-	-
8	**+**	**-**	**-**	**-**	**-**	**-**	**+**
10	+	-	+	-	-	-	+
11	+	-	-	-	-	-	-
12	+	-	+	-	-	-	+
13	+	-	-	-	-	-	-
15	+	-	-	-	-	-	-
17	+	-	-	-	-	-	-
19	+	-	+	-	-	-	-
20	+	-	-	-	-	-	-
23	+	-	-	-	-	-	-
24	+	-	-	-	-	-	-
25	+	-	-	-	-	-	-
28	+	-	+	+	-	-	-
29	+	-	-	-	-	-	+
30	+	-	+	-	-	-	-
32	+	-	-	-	-	-	+
34	+	-	-	-	-	-	+
*PC	+	+	+	+	+	+	+

PC: positive control

Figure 1: RT-PCR threshold curves for the amplification the genes of sexually transmitted bacteria
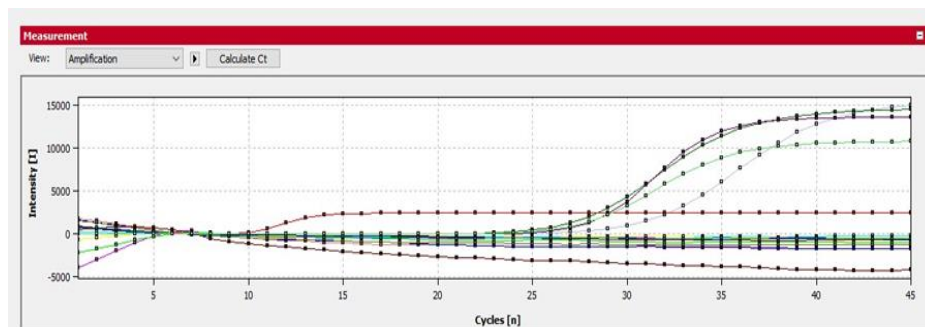

Figure 2: RT-PCR threshold curves for the amplification of genes of sexually transmitted bacteria

Nonpregnant treated with or azithromycin & pregnant women with Ureaplasma infection were treated to reduce complications; Ureaplasma in this study was treated with azithromycin, and pregnant women who have premature rupture of the membrane were treated with getting azithromycin, clarithromycin, and erythromycin. Therefore, this reduces the risk of Ureaplasma infection in newborns; While chlamydia results from chlamydia trachomatis and genitalium, the prevalence in this study was 5.8% and 2.8%, respectively. Chlamydia, in some cases in this study, does not produce symptom but cause infertility; some cases cause burning pain during urination, odorless yellow or cloudy vaginal discharge, and salpingitis. Also, the infection can be transmitted to the features and cause newborn ophthalmia neonatorum. So, the treatment is important. This study uses a single 1-g dose of azithromycin or doxycycline with a dose of about 100mg orally twice per day for a weak person. In pregnant women, the recommended treatment is 500 mg of erythromycin or amoxicillin four times per day orally. While resistance-guided therapy, such as doxycycline 100 mg orally twice daily for seven days followed by moxifloxacin 400 mg orally once daily for seven days, was used in this trial to treat M. genitalium, and doxycycline is used as the first line of treatment because it lowers the organism burden and helps the organism recover. This therapy is in line with the suggested therapy [35], which states that doxycycline has a cure rate of 30–40%, but resistance does not increase. Azithromycin has a cure rate of 85–95% in macrolide-susceptible infections. Pre-treatment with doxycycline may lessen organism load and the possibility of macrolide resistance selection, and a prolonged course of azithromycin seems to have a greater cure rate. Moxifloxacin is a second-line treatment option, although resistance is growing.

The most widely used tests today are DNA detection assays, particularly PCR technology, because it quickly and concurrently identifies many diseases. Therefore, PCR tests eliminate individual detections, allow faster and more accurate detections, and lower labor and reagent costs, particularly for bacteria that cause sexually transmitted diseases [36].

The researcher [37] employed the RT-PCR technique to detect Mycoplasma genitalium, the cause of non-gonococcal urethritis, sending complementary amplification primers to all pertinent variants of the 23s rRNA gene often discovered. Furthermore, using multiplex PCR, the scientists found cases of genital Mycoplasma [38].

The current study showed a low rate of all types of sexually transmitted bacteria compared with other studies in various countries, including the Middle East, North Africa and the Eastern Mediterranean, where sexually transmitted diseases are spread in greater proportions for C. trachoma and N. Gonorrhea [39].

Furthermore, the prevalence rates of these diseases are higher in the United Kingdom, France, and the American regions [40]. The low rate of prevalence of this infection in Iraq may be due to the embrace of the majority of its population to the religion of Islam and adherence to religious customs and beliefs.

Conclusion

Our results supported the need for greater STI prevention knowledge. Seven STI pathogens were successfully identified in our research population using the real-time PCR approach. The *Mycoplasma genitalium* pathogen had the lowest detection rates compared to the typically high Ureaplasma parvum pathogen detection rates. The findings of this study should prompt medical practitioners to establish affordable, universally available reproductive healthcare services. In addition, public education programs are required to encourage people to practice healthy sexual conduct.

Acknowledgments

Funding

Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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