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Detection of sexually transmitted infection agents in pregnant women using multiplex polymerase chain reaction method

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Abstract

Background Sexually transmitted infections (STIs) are a significant public health concern that can lead to serious outcomes such as infertility, pregnancy complications, and neonatal infections. This study aimed to evaluate the prevalence of STI and their associated risk factors in symptomatic and asymptomatic pregnant women.

Methods Between July and October 2024, a total of 300 pregnant women in their third trimester, including 113 symptomatic and 187 asymptomatic individuals aged 18 to 45 years, who sought antenatal care at the Gynecology and Obstetrics Clinic of Ankara Etlik City Hospital, were included in the study. The detection of STIs agents in vaginal swab samples was performed using multiplex polymerase chain reaction in the Molecular Diagnosis Laboratory of the Department of Microbiology.

Results The overall prevalence of STIs was 34.3% (103/300), with single and multiple infections accounting for 28.3% and 6.0% of cases, respectively. The most frequently detected pathogens were *Ureaplasma parvum/urealiticum* (29.0%), *Mycoplasma hominis* (4.6%), and *Chlamydia trachomatis* (2.3%). Co-infections were commonly observed between *Ureaplasma parvum/urealiticum* and *Mycoplasma hominis*. No significant difference in STI prevalence was observed between the symptomatic (35.4%) and asymptomatic (33.7%) groups. Co-infection with non-STI bacterial agents, such as *Gardnerella vaginalis* and *Streptococcus agalactiae*, increased the risk of STIs by 1.96 times ($p=0.006$).

Conclusions This study revealed that STIs occur at similar rates among symptomatic and asymptomatic pregnant women. This finding highlights the critical importance of detecting asymptomatic cases to prevent the spread of silent infections and to safeguard maternal and neonatal health. *Ureaplasma parvum/urealiticum* were identified as the most common pathogens. Given that co-infections with non-STI bacterial agents significantly increase the risk of STIs, multiplex PCR-based multicenter and prospective studies are essential to refine screening strategies for pregnant women.

Keywords Sexually transmitted infections, Pregnancy, Multiplex PCR, Prevalence

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Introduction

Sexually transmitted infections (STIs) are a global health concern, affecting millions annually and causing severe complications such as infertility, pregnancy-related issues, and neonatal infections. According to the World Health Organization (WHO), approximately 376 million new STI cases occur globally each year [1]. Factors such as socioeconomic disparities, limited healthcare access, low education levels, and inadequate preventive measures contribute to their prevalence. Additionally, risky sexual behaviors further amplify transmission rates [2, 3].

During pregnancy, STIs can lead to complications such as preterm birth, low birth weight, and congenital infections [4, 5]. Agents primarily transmitted through sexual contact include *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Trichomonas vaginalis*, *Ureaplasma parvum/urealiticum*, *Haemophilus ducreyi*, Herpes simplex virus (HSV)-1, and HSV-2 [6–11]. According to the European STIs Guidelines, most cases of *M. hominis* and *Ureaplasma* species colonization occur through sexual contact and are correlated with the number of sexual partners [7]. *T. pallidum* causes congenital infection, whereas HSV, *N. gonorrhoeae*, and *C. trachomatis* lead to perinatal infections. The primary risk associated with HSV, *N. gonorrhoeae*, and *C. trachomatis* is perinatal infection related to transmission during labor [6]. *Gardnerella vaginalis* and *Streptococcus agalactiae* are microorganisms that are not primarily classified as sexually transmitted pathogens but can be part of the vaginal flora or act as opportunistic agents causing infections. *G. vaginalis* is associated with bacterial vaginosis, while *S. agalactiae* is linked to neonatal sepsis, meningitis, and maternal infections. Both bacteria can manifest in symptomatic and asymptomatic forms and have been associated with adverse pregnancy outcomes [8–11].

Molecular diagnostic methods like multiplex polymerase chain reaction (PCR) provide rapid and accurate detection of STIs, surpassing traditional techniques such as serological tests and cultures [12–14]. Multiplex PCR enables high-sensitivity detection of genital pathogens from various sample types, including swabs and urine samples, with the ability to detect multiple agents in a single test [15].

This study aimed to determine the prevalence of 10 different bacterial, viral, and parasitic STI agents, along with *G. vaginalis* and *S. agalactiae*, in symptomatic and asymptomatic pregnant individuals in the third trimester using multiplex PCR. Additionally, we investigated their associations with demographic characteristics and risk factors.

Materials and methods

Between July 2024 and October 2024, 300 pregnant women aged between 18 and 45 years in their third trimester who sought antenatal care at the Gynecology and Obstetrics Clinic of Ankara Etlik City Hospital were included in the study. The identification of STIs in vaginal swab samples using multiplex PCR was performed in the Molecular Diagnosis Laboratory of the Department of Microbiology. The study population was divided into two groups: symptomatic and asymptomatic pregnant women. The symptomatic pregnant group was composed of women who exhibited at least one of the following symptoms: vaginal discharge, vaginal itching, dysuria, inguinal lymphadenopathy, genital ulcers, or dyspareunia. The asymptomatic group consisted of individuals who requested testing as part of a general health assessment. A total of 113 women suspected of having STIs during follow-up were enrolled in the symptomatic group, while the control group consisted of 187 asymptomatic pregnant women. Age groups were categorized as 18–25, 26–35, and 36–45 years to assess STI prevalence across different age ranges.

During the four-month study period, vaginal swab samples were collected from all participants by specialized physicians. Samples were obtained using vaginal swab following the manufacturer's instructions and stored in specialized transport tubes designed to preserve pathogen stability (VNAT, Bioeksen R&D Technologies Inc., Istanbul, Türkiye). After collection, the samples were transported to the laboratory under appropriate conditions and stored at +4 °C for multiplex PCR analysis on the same day. The samples were directly subjected to multiplex PCR without additional extraction processes.

The age, ethnicity (Turkish or foreign national), educational level (no formal education, primary, middle, or high school), condom use, presence of chronic disease (diabetes mellitus), immunosuppression status, history of malignancy, parity, gravida, and history of miscarriage of pregnant women were recorded. Additionally, pregnant women were asked about their history of hepatitis B, hepatitis C, human immunodeficiency virus (HIV), human papillomavirus (HPV), and syphilis.

Criteria for inclusion /exclusion

Pregnant women aged between 18 and 45 years who were in their third trimester and reported at least one characteristic symptom of STIs, as well as asymptomatic pregnant women who requested testing as part of a general health assessment, were included in the study, provided they consented to participate. In cases of multiple visits, only the first collected sample was considered for evaluation. Pregnant women who were not in their third trimester, outside the 18–45 age range, or had received antibiotic treatment (oral or vaginal ovules) within the

past two weeks were excluded from the study. Additionally, those experiencing preterm labor or other obstetric complications, as well as those with active vaginal bleeding at the time of sample collection, were not included.

Preprocessing of clinical samples and multiplex PCR analysis

Swab samples in VNAT collection tubes were brought to room temperature and thoroughly mixed by vortexing. Real-time multiplex PCR amplification of 12 microorganisms, including *C. trachomatis*, HSV-1, HSV-2, *H. ducreyi*, *M. hominis*, *M. genitalium*, *N. gonorrhoeae*, *T. pallidum*, *Ureaplasma* spp., *T. vaginalis*, *G. vaginalis* and *S. agalactiae*, was performed using the Bio-Speedy® Sexually Transmitted Infection RT-qPCR Kit (Biospeedy®, Bioeksen R&D Technologies Inc., Istanbul, Türkiye) on a Mic PCR thermocycler device (Bio Molecular Systems, Australia), following the manufacturer's protocol. Primers specifically designed to target the gene regions of each microorganism were used for PCR analyses (Table 1). Positive and negative controls for all targeted agents were included in each run to ensure accuracy. The results were analyzed using Sigmoida Software (V 8.6 REV.56) by Bioeksen, following the manufacturer's guidelines.

The analytical sensitivity of the PCR kit is 98.75%, and the specificity is 99.28% (Catalog No: BS-STI-L-25/BS-STI-L-100, Revision Date: 24-06-2024/Rev.02).

Statistical analysis

Statistical analysis was conducted using SPSS 25 (IBM Corp., Armonk, NY, USA). Data normality was evaluated with the Kolmogorov-Smirnov test. Descriptive statistics were used to summarize variables: qualitative data were presented as frequencies (%) and quantitative data as medians with interquartile ranges (IQR, range between the 25th to 75th percentiles).

Table 1 Multiplex PCR targets

Target species	Target gene
Bacterial panel	
Streptococcus agalactiae	*gfb*
Neisseria gonorrhoeae	*ompC, por A*
Treponema pallidum	*MSP porin*
Mycoplasma genitalium	*mgpA*
Mycoplasma hominis	*gap*
Chlamydia trachomatis	*DNA B*
Haemophilus ducreyi	*recC*
Viral panel	
Herpes simplex virus-1	*US5*
Herpes simplex virus-2	*US3*
Parasite panel	
Trichomonas vaginalis	*TVAGG3_0181000*

**Ureaplasma parvum/urealiticum*

Categorical variables were compared using Pearson's chi-square or Fisher's exact test. Numerical variables were analyzed using the Mann-Whitney U test (two groups) or the Kruskal-Wallis test (more than two groups).

Binary logistic regression identified STI risk factors, including age, ethnicity, education, contraceptive use, bacterial co-infections, pregnancies, and deliveries. Odds ratios (ORs) were calculated, with statistical significance set at $p < 0.05$.

Results

Study participants' characteristics

A total of 300 women participated in the study, with an age range of 18–41 years and a mean age of 26 years (IQR: 23–30). Among the participants, 37.7% ($n = 113$) reported at least one symptom. The median ages of symptomatic and asymptomatic pregnant women were 27 years (IQR: 24–30) and 26 years (IQR: 23–31), respectively, with no statistically significant difference ($p = 0.804$).

There were no significant differences between symptomatic and asymptomatic women in terms of ethnicity or educational level ($p = 0.287$ and $p = 0.801$, respectively). All participants were married, and only 24% had attained a higher education.

All women were in their third trimester of pregnancy, with the majority (43.7%) experiencing their first pregnancy. None of the pregnant women reported having HIV, HPV, hepatitis B, or hepatitis C virus. Additionally, no history of diabetes mellitus, immunosuppression, or malignancy was identified.

The sociodemographic and obstetric characteristics of the participants are detailed in Table 2.

General prevalence of STI agents

The overall prevalence of infection with at least one STI pathogen among the participants was 34.3% (95% CI: 28.9–40.0). The prevalence of single infections was 28.3% (95% CI: 23.3–33.8), while multiple infections were identified in 6.0% (95% CI: 3.5–9.3) of cases. The most common STI agent detected among pregnant women was *Ureaplasma* spp., found in 29.0% ($n = 87$) of the participants, followed by *M. hominis* (4.6%, $n = 14$) and *C. trachomatis* (2.3%, $n = 7$). The most frequent co-infection involved *Ureaplasma* spp. and *M. hominis*, observed in 2.7% (8/300) of cases. None of the clinical samples tested positive for *N. gonorrhoeae*, *T. pallidum*, *M. genitalium*, or *H. ducreyi*.

Among the study participants, 12.3% (37/300) were foreigners. *Ureaplasma* spp. was the most commonly identified agent in both Turkish participants (29.2%, 77/263) and foreign nationals (27%, 10/37), with no significant difference in prevalence between the two groups ($p = 0.864$) (Table 3).

Table 2 Sociodemographic, clinic and obstetric characteristics of study participants

Characteristics	Total		Symptomatic group		Asymptomatic group		*p* value
	N	%	N	%	N	%	
Age, years, median(IQR)	26	(23–30)	27	(24–30)	26	(23–31)	0.804
Age group (years)							
18–25	130	(43.3)	52	(46.0)	78	(41.7)	
26–35	146	(48.7)	51	(45.1)	95	(50.8)	0.628
36–45	24	(8.0)	10	(8.8)	14	(7.5)	
Etnicity							
Turkish	263	(87.7)	102	(90.3)	161	(86.1)	0.287
Foreign national	37	(12.3)	11	(9.7)	26	(13.9)	
Location							
Urban	298	(99.3)	111	(100)	187	(100)	0.141
Rural	2	(0.7)	2	(0)	0	(0)	
Education							
No formal education	25	(8.3)	10	(8.8)	15	(8.0)	
Primary	66	(22.0)	20	(17.7)	46	(24.6)	**0.034**
Middle	137	(45.7)	63	(55.8)	74	(39.6)	
High-school	72	(24.0)	20	(17.7)	52	(27.8)	
Gravida							
Primigravida	131	(43.7)	48	(42.5)	83	(44.4)	
Second	84	(28.0)	29	(25.7)	55	(29.4)	0.547
Third and onwards	85	(28.3)	36	(31.9)	49	(26.2)	
History of STI							
Non	299	(99.7)	113	(100)	186	(99.5)	0.999
Yes	1	(0.3)	0	(0)	1	(0.5)	
Condom use							
Non	286	(95.3)	108	(95.6)	178	(95.2)	0.877
Yes	14	(4.7)	5	(4.4)	9	(4.8)	

IQR, interquartil range; STI, sexually transmitted infection

Distribution of STI agents by age groups

Across all age groups, *Ureaplasma* spp. was the most common STI agent. The highest positivity rate for *Ureaplasma* spp. was observed in the 36–45 years age group (41.7%), although this difference was not statistically significant ($p = 0.113$). No significant differences were detected in the distribution of STI agents across the age groups (Table 4).

Among the 103 women with positive STI results, 63 (61.2%) were asymptomatic. In the symptomatic group, the prevalence of at least one positive agent was 35.4% (40/113, 95% CI: 26.6–44.9), while in the asymptomatic group, it was 33.7% (63/187, 95% CI: 26.9–40.9), with no statistically significant difference ($p = 0.763$) (Table 5).

The most frequently detected agents in the symptomatic group were *Ureaplasma* spp. (30.1%), HSV-1 (2.7%), *M. hominis* (2.7%), HSV-2 (1.8%), and *C. trachomatis* (0.9%). In the asymptomatic group, the most common pathogens were *Ureaplasma* spp. (28.3%), *M. hominis* (5.9%), and *C. trachomatis* (3.2%). A comparison of agent distribution between symptomatic and asymptomatic groups showed no significant differences. Single infections were more prevalent in both groups ($p = 0.063$). Additionally, *Ureaplasma* spp. infections were detected at similar frequencies in the symptomatic (30.1%) and asymptomatic (28.3%) groups ($p = 0.747$) (Table 5).

Prevalence of non- STI agents with clinical significance in pregnant women

In this study, *S. agalactiae* and *G. vaginalis*, which are not sexually transmitted but can lead to clinically significant outcomes in pregnant women, were also investigated. Among all participants, *S. agalactiae* was identified in 3.3% (10/300) and *G. vaginalis* in 35.7% (107/300).

Of the 107 participants infected with *G. vaginalis*, 54% were found to have a single pathogen, while 46% exhibited co-infections. *Ureaplasma* spp. co-infection was found to be the most common in both symptomatic and asymptomatic pregnant women and demonstrated a statistically significant difference ($p = 0.029$) (Table 6).

The prevalence of *G. vaginalis* was higher in the symptomatic group (39.8%, 45/113) compared to the asymptomatic group (33.2%, 62/187); however, the difference was not statistically significant ($p = 0.243$). *S. agalactiae* was observed at similar rates in the symptomatic (2.7%, 3/113) and asymptomatic (3.7%, 7/187) groups, with no significant difference ($p = 0.748$). Among the 10 women with *S. agalactiae*, it was detected as the sole pathogen in

Table 3 Laboratory features of the patients

	Total	Turkish	Foreign national	*p* value
	N (%)	N (%)	N (%)	
No. of pregnant women	300 (100)	263 (87.7)	37 (12.3)	
STI status				
Absent	197 (65.7)	171 (65.0)	26 (70.3)	
Present	103 (34.3)	92 (35.0)	11 (29.7)	0.528
Infection agents				
Ureaplasma spp.	87 (29.0)	77 (29.2)	10 (27.0)	
Mycoplasma hominis	14 (4.6)	12 (4.6)	2 (5.4)	
HSV-1	8 (2.7)	8 (3.0)	0 (0)	
Chlamydia trachomatis	7 (2.3)	6 (2.2)	1 (2.7)	0.864
Herpes simplex virus-2	4 (1.3)	4 (1.5)	0 (0)	
Trichomonas vaginalis	1 (0.3)	1(0.4)	0 (0)	
Infection type				
Single infection	85 (28.3)	76 (28.9)	9 (24.3)	
Ureaplasma spp.	71 (23.7)	63 (24.0)	8 (21.6)	
HSV-1	5 (5.7)	5 (1.9)	0 (0)	
Mycoplasma hominis	4 (1.3)	4 (1.5)	0 (0)	
Chlamydia trachomatis	3 (1.0)	2 (0.8)	1 (2.7)	
Herpes simplex virus-2	2 (0.7)	2 (0.8)	0 (0)	
Multiple infection	18 (6.0)	16 (6.1)	2 (5.4)	
Ureaplasma spp., *Mycoplasma hominis*	8 (2.7)	6 (2.3)	2 (5.4)	
Chlamydia trachomatis, *Ureaplasma* spp.	3 (1.0)	3 (1.1)	0 (0)	
HSV-1, *Ureaplasma* spp.	3 (1.0)	3 (1.1)	0 (0)	
HSV-2, *Ureaplasma* spp.	2 (0.7)	2 (0.8)	0 (0)	
Chlamydia trachomatis, *Mycoplasma hominis*	1 (0.3)	1 (0.4)	0 (0)	
Mycoplasma hominis, *Trichomonas vaginalis*	1 (0.3)	1 (0.4)	0 (0)	

STI, sexually transmitted infection; HSV-1, Herpes simplex virus-1; HSV-2, Herpes simplex virus-2; *Ureaplasma parvum/urealiticum*, *Ureaplasma* spp; N, number

five cases (50%). In the remaining five cases, it was found alongside HSV-1 ($n=1$), *Ureaplasma* spp. ($n=2$), and *G. vaginalis* ($n=2$).

Risk factors

Co-infection with *G. vaginalis* significantly increased the risk of developing STIs (OR: 1.96, 95% CI: 1.20–3.19, $p=0.006$). Although an increased relative risk for STIs was observed among women under the age of 36, those with no formal education or only primary education, primiparous or multiparous women, and multigravida women, these associations were not statistically significant. Additionally, contraceptive status and ethnicity were not associated with STIs. The risk factors for STIs in pregnant women are summarized in Table 7.

Table 4 Distribution of STI agents by age groups

	Age groups			*p* value
	18–25	26–35	36–45	
	N (%)	N (%)	N (%)	
No. of pregnant women	130 (43.3)	146 (48.7)	24 (8.0)	
HSV-1	4 (3.1)	3 (2.1)	1 (4.2)	0.533
HSV-2	1 (0.8)	3 (2.1)	0 (0)	0.999
Neisseria gonorrhoeae	0 (0)	0 (0)	0 (0)	-
Treponema pallidum	0 (0)	0 (0)	0 (0)	-
Ureaplasma spp.	42 (32.3)	35 (24.0)	10 (41.7)	0.113
Mycoplasma genitalium	0 (0)	0 (0)	0 (0)	-
Mycoplasma hominis	4 (3.1)	10 (6.8)	0 (0)	0.176
Chlamydia trachomatis	4 (3.1)	3 (2.1)	0 (0)	0.839
Haemophilus ducreyi	0 (0)	0 (0)	0 (0)	-
Trichomonas vaginalis	0 (0)	1 (0.7)	0 (0)	0.999

STI, sexually transmitted infection; HSV-1, Herpes simplex virus-1; HSV-2, Herpes simplex virus-2; *Ureaplasma parvum/urealiticum*, *Ureaplasma* spp; N, number

Table 5 Distribution of infectious agents based on groups

	Symptomatic group	Asymptomatic group	*p* value
	N (%)	N (%)	
STI status			
Absent	73 (64.6)	124 (66.3)	0.763
Present	40 (35.4)	63 (33.7)	
Infectious agents			
HSV-1	3 (2.7)	5 (2.7)	0.999
HSV-2	2 (1.8)	2 (1.1)	0.634
Ureaplasma spp.	34 (30.1)	53 (28.3)	0.747
Mycoplasma hominis	3 (2.7)	11 (5.9)	0.199
Chlamydia trachomatis	1 (0.9)	6 (3.2)	0.261
Trichomonas vaginalis	0 (0)	1 (0.5)	0.999
Infection type			
Single infection	37 (32.7)	48 (25.7)	**0.063**
Multiple infection	3 (2.7)	15 (8.0)	
Details of single and multiple infections			
Ureaplasma spp.	31 (27.4)	40 (27.3)	0.200
Chlamydia trachomatis	1 (0.9)	2 (1.1)	0.999
HSV-1	2 (1.8)	3 (1.6)	0.999
HSV-2	1 (0.9)	1 (0.5)	0.999
Mycoplasma hominis	2 (1.8)	2 (1.1)	0.640
Chlamydia trachomatis, *Mycoplasma hominis*	0 (0)	1 (0.5)	0.999
Chlamydia trachomatis, *Ureaplasma* spp.	0 (0)	3 (1.6)	0.280
HSV-1, *Ureaplasma* spp.	1 (0.9)	2 (1.1)	0.999
HSV-2, *Ureaplasma* spp.	1 (0.9)	1 (0.5)	0.999
Mycoplasma hominis, *Trichomnas vaginalis*	0 (0)	1 (0.5)	0.999
Mycoplasma hominis, *Ureaplasma* spp.	1 (0.9)	7 (3.7)	0.146

N, number; STI, sexually transmitted infection; HSV-1, Herpes simplex virus-1; HSV-2, Herpes simplex virus-2; *Ureaplasma parvum/urealiticum, Ureaplasma* spp.

Table 6 Co-infections of *G. vaginalis* (*n*=107)

	Total	Symptomatic group	Asymptomatic group	*p* value
	***N* (%)**	***N* (%)**	***N* (%)**	
Single infection	58 (54)	27 (60)	31 (50)	0.305
Multiple infection	49 (46)	18 (40)	31 (50)	
Ureaplasma spp.	33 (30)	15 (33.3)	18 (29)	**0.029***
Ureaplasma spp., *Mycoplasma hominis*	5 (4.6)	1 (2.2)	4 (6.4)	
Mycoplasma hominis	3 (4.6)	1 (2.2)	2 (3.2)	
HSV-1, *Ureaplasma* spp.	2 (1.8)	0 (0)	2 (3.2)	
Streptococcus agalactia	2 (1.8)	0 (0)	2 (3.2)	
Chlamydia trachomatis	1 (0.9)	1 (2.2)	0 (0)	
HSV-1	1 (0.9)	0 (0)	1 (1.6)	
Ureaplasma spp., *Chlamydia trachomatis*	1 (0.9)	0 (0)	1 (1.6)	
Ureaplasma spp., HSV-2	1 (0.9)	0 (0)	1 (1.6)	

N, number; HSV-1, Herpes simplex virus-1; HSV-2, Herpes simplex virus-2; *Ureaplasma parvum/urealiticum*, *Ureaplasma* spp.

*Comparison of *Ureaplasma* spp. co-infection with other multiple infection types.

Table 7 Risk factors of STI in pregnant women

Risk factor	STI		OR	OR	*p* value
	Negative (*N*)	Positive (*N*)		(95%CI)	
Age groups (years)					
18–25	82	48	1.22	0.50–2.96	0.659
26–35	101	45	1.60	0.66–3.88	0.295
36–45	14	10	Reference		
Etnicity					
Turkish	171	92	Reference		
Foreign national	26	11	0.78	0.37–1.66	0.529
Education (years)					
No formal education	17	8	1.64	0.60–4.51	0.331
Primary	43	23	1.87	0.88–3.97	0.102
Middle	81	56	0.77	0.42–1.42	0.409
High-school	56	16	Reference		
Contraception					
No	189	97	0.68	0.23–2.02	0.493
Condom use	8	6	Reference		
Non-STIs*					
Absent	133	53	Reference		
Present	64	50	1.96	1.20–3.19	**0.006**
Gravida					
Primigravida	98	43	Reference		
Multigravida	99	60	1.38	0.85–2.23	0.188
Parity					
Nullipar	102	42	Reference		
Primipar/multipar	95	61	1.55	0.96–2.52	0.070

STI, sexually transmitted infection; OR, Odds ratio; CI, confidence interval; N, number

*Non-STIs: *Gardnerella vaginalis* and/or *Streptococcus agalactiae* positivity

Discussion

In this study, the prevalence of STIs was found to be 34.3% among pregnant women, with at least one STI pathogen detected. Although the prevalence was slightly higher in symptomatic pregnant women (35.4%) compared to asymptomatic women (33.7%), this difference was not found to be statistically significant. The similar rate in asymptomatic cases demonstrates the silent progression of these infections. The literature also highlights the crucial role of asymptomatic infections in STI transmission [16, 17]. STIs in pregnant women are often asymptomatic but can pose serious risks if left untreated. However, studies using multiplex PCR to assess STI prevalence in this group remain limited [18]. For instance, a

study conducted in Brazil involving 400 pregnant women (40.3% asymptomatic) reported a 24% prevalence of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*, with 59% of cases being asymptomatic [4]. Similarly, another study of 2,728 pregnant women found a 21% prevalence of at least one STI, including *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. hominis* [19]. In South Africa, the prevalence of seven STI pathogens in asymptomatic pregnant women attending antenatal care was investigated using a multiplex PCR test. Of the 202 participants tested, 88 (43.5%) were found to be positive for at least one STI pathogen. *U. urealyticum* (43%), *C. trachomatis* (41%), and *T. vaginalis* (10%) were identified as the most common pathogens. *M. genitalium* (5%) and *N. gonorrhoeae* (2%) were detected less frequently [20]. These regional differences in STI prevalence may result from variations in disease distribution patterns; demographic characteristics such as age, education, and income levels; a high proportion of asymptomatic cases; limited access to healthcare services; risky sexual behaviors; cultural norms; and gaps in health policies and screening guidelines [3, 19–23].

The diagnosis of STIs requires rapid, reliable, and effective diagnostic methods to ensure positive outcomes for both individual and public health. As a result, there has been a significant global increase in the use of PCR-based methods [18]. The prevalence of *Ureaplasma* spp. in pregnant women varies widely (8–93%) depending on diagnostic methods, highlighting its global presence [24]. In this study, *Ureaplasma* spp. was the most common STI pathogen, followed by *C. trachomatis* and *M. hominis*, with co-infection of *Ureaplasma* spp. and *M. hominis* being the most frequent. This contrasts with findings from other PCR-based studies [19–21]. The variability of detected infectious agents depends on factors such as geographical location, population characteristics, individual behaviors, diagnostic methods used, the sample collection process, and the design of multiplex PCR kits. Disease prevalence may vary by region, and access to healthcare services can differ. Population characteristics include age, immune status, and socioeconomic factors, while behavioral factors encompass sexual practices and contraceptive methods. Additionally, the sensitivity of diagnostic techniques, sampling methods, and the pathogens targeted in PCR kits can directly influence infection detection rates [25–27]. Although *Ureaplasma* spp. is part of the normal genital flora, it is also associated with infertility and non-gonococcal urethritis. In vitro studies have shown that *U. parvum* forms biofilms, contributing to chronic and mixed bacterial infections. Additionally, *Ureaplasma* spp. has been linked to pregnancy complications such as premature rupture of membranes, amniotic fluid infections, preterm birth, and low birth weight [28–36].

Similarly, *G. vaginalis* disrupts the vaginal microbiota, increasing the risk of pregnancy complications such as preterm birth, low birth weight, and puerperal infections, as well as STIs [37–39]. In this study, *G. vaginalis* was detected in 35.7% of cases, with co-infections (primarily with *Ureaplasma* spp.) in 46%, suggesting its role in altering the vaginal microecosystem and facilitating the colonization of other pathogens. This study also found that non-STI bacterial co-infections, particularly with *G. vaginalis*, increased the risk of STIs by 1.96 times. The most common co-infection observed was between *G. vaginalis* and *Ureaplasma* spp. Numerous studies have highlighted a strong association between *G. vaginalis* and STIs [40–43]. Recent research suggests that disruptions in normal genital flora, including co-infections involving *Ureaplasma* spp. and *G. vaginalis*, may impair fertility [44]. Although these microorganisms are common in sexually active individuals as part of the commensal flora, their pathogenic roles remain unclear, emphasizing the need for further investigation [44, 45]. Treating non-STI bacterial infections in pregnant women may help prevent STIs and adverse pregnancy outcomes.

One of the strengths of this study was its comparison of both symptomatic and asymptomatic groups. Furthermore, the use of a highly sensitive diagnostic method, such as multiplex PCR, enhances the reliability of the findings. Nevertheless, the study has certain limitations, including its inability to examine associations with clinical outcomes and its single-center design, which restricted the evaluation of regional variations. In addition, *G. vaginalis* was analyzed as part of the multiplex PCR panel, but Nugent scoring or Amsel criteria were not applied for the diagnosis of bacterial vaginosis.

Sensitive, rapid, and reliable tests are essential for the early diagnosis of genital infections, as traditional methods have low sensitivity and fail to detect certain pathogens. Compared to conventional techniques, multiplex PCR is more effective and also reduces treatment costs [3, 4, 45–47].

In conclusion, this study revealed that the prevalence of STIs was similar between symptomatic and asymptomatic pregnant women. *Ureaplasma* spp. was the most frequently detected pathogen, and co-infections with non-STI bacterial infections significantly increased STI risk. Therefore, it is crucial to reassess screening strategies for pregnant women. To further evaluate these findings, multicenter prospective studies are recommended.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was conducted after obtaining ethical approval from the Non-Interventional Scientific Research Committee of the Scientific Research Evaluation and Ethics Board of Ankara Etlik City Hospital (approval number: AEŞH-BADEK-2024-392). The research was carried out in accordance with the principles outlined in the Declaration of Helsinki, and informed consent was obtained from all participants.

Consent for publication
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The authors declare no competing interests.

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