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Clinical Signs and Symptoms in Sexually Transmitted Infections Confirmed by Multiplex PCR: Practical Tips for Clinicians

Mehmet Ezer[1], **İsmet Bilger Erihan**[1], **Samet Kırat**[2], **Gülfem Nur Yıldız**[3], **Murat Karameşe**[3]

1 Kafkas University, Medical School, Department of Urology, 36100, Kars, Türkiye

2 Kafkas University, Medical School, Department of Gynecology and Obstetrics, 36100, Kars, Türkiye

3 Kafkas University, Medical School, Department of Medical Microbiology, 36100, Kars, Türkiye

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Abstract

Objective: Sexually transmitted infections (STIs) pose significant global health challenges due to their often-asymptomatic nature and associated complications. In urological practice, early and accurate diagnosis is essential to prevent sequelae such as chronic prostatitis, urethral strictures, and infertility. Molecular diagnostic methods, particularly Multiplex PCR, offer rapid and sensitive detection of multiple pathogens.

Methods: This retrospective study included 194 patients who presented with urogenital symptoms to the Urology and Obstetrics-Gynecology outpatient clinics of a tertiary care university hospital. Urethral and cervical/vaginal swab samples were collected and analyzed using a Multiplex PCR panel targeting 12 STI pathogens. Demographic and clinical data were recorded, and statistical analysis was performed to evaluate symptom-pathogen correlations.

Results: Of the participants, 73.2% were male, with a mean age of 43 years. Multiplex PCR detected at least one pathogen in 40.2% of cases, with *Ureaplasma parvum*, *Gardnerella vaginalis*, and *Haemophilus ducreyi* being the most common. Significant correlation was found between some clinical symptoms and PCR positivity. A considerable proportion of positive cases were asymptomatic, highlighting the limitations of symptom-based diagnosis.

Conclusion: Multiplex PCR significantly enhances diagnostic accuracy in STI management within urology. Early molecular detection enables targeted therapy, reduces unnecessary antibiotic use, and helps prevent long-term complications. The high rate of asymptomatic infections underscores the necessity for routine molecular screening, especially in high-risk populations. Further research should focus on expanding diagnostic panels and evaluating the cost-effectiveness of molecular testing strategies.

Keywords: Sexually transmitted infections, Multiplex PCR, *Ureaplasma parvum*, *Gardnerella vaginalis*

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Correspondence / Yazışma Adresi: *Murat Karameşe, Kafkas University, Faculty of Medicine Department of Medical Microbiology 36100, Kars, Türkiye e-mail: murat_karamese@hotmail.com*

Multiplex PCR ile Tanılanan Cinsel Yolla Bulaşan Enfeksiyonlarda Klinik Bulgular ve Semptomlar: Klinisyenler için Pratik Öneriler

Öz

Amaç: Cinsel yolla bulaşan enfeksiyonlar (CYBE), sıklıkla asemptomatik seyretmeleri ve neden oldukları komplikasyonlar nedeniyle küresel ölçekte önemli bir halk sağlığı sorunu oluşturmaktadır. Ürolojik pratikte erken ve doğru tanı, kronik prostatit, üretral darlıklar ve infertilite gibi sekellerin önlenmesi açısından kritik öneme sahiptir. Moleküler tanı yöntemleri, özellikle Multiplex PCR, birden fazla patojeni hızlı ve yüksek duyarlılıkla tespit etme imkânı sunmaktadır.

Yöntemler: Bu retrospektif çalışmaya, üçüncü basamak bir üniversite hastanesinin Üroloji ile Kadın Hastalıkları ve Doğum polikliniklerine ürogenital semptomlarla başvuran 194 hasta dâhil edildi. Üretral ve servikal/vajinal sürüntü örnekleri alındı ve 12 CYBE etkenini hedefleyen Multiplex PCR paneli ile etkenler tespit edildi. Hastalara ait demografik ve klinik veriler kaydedildi; semptom–patojen ilişkisini değerlendirmek amacıyla istatistiksel analizler gerçekleştirildi.

Bulgular: Katılımcıların %73,2'si erkek olup ortalama yaş 43 idi. Multiplex PCR ile olguların %40,2'sinde en az bir patojen tespit edildi. En sık saptanan patojenler *Ureaplasma parvum*, *Gardnerella vaginalis* ve *Haemophilus ducreyi* idi. Bazı klinik semptomlar ile PCR pozitifliği arasında anlamlı korelasyon tespit edildi. Pozitif vakaların önemli bir kısmının asemptomatik olduğu belirlendi; bu durum, semptom temelli tanının sınırlılıklarını ortaya koymaktadır.

Sonuç: Multiplex PCR, ürolojide CYBE yönetiminde tanısal doğruluğu belirgin şekilde artırmaktadır. Erken moleküler tanı, hedefe yönelik tedaviye olanak tanır, gereksiz antibiyotik kullanımını azaltır ve uzun dönem komplikasyonların önlenmesine katkı sağlar. Asemptomatik enfeksiyonların yüksek oranı, özellikle yüksek riskli popülasyonlarda rutin moleküler taramanın gerekliliğini vurgulamaktadır. Gelecek çalışmalar, tanı panellerinin genişletilmesi ve moleküler test stratejilerinin maliyet etkinliğinin değerlendirilmesine odaklanmalıdır.

Anahtar kelimeler: Cinsel yolla bulaşan enfeksiyonlar, Multiplex PCR, *Ureaplasma parvum*, *Gardnerella vaginalis*.

INTRODUCTION

Sexually transmitted infections (STIs) represent a group of infectious diseases caused by various microorganisms and are primarily transmitted through unprotected sexual contact, resulting in significant global morbidity and mortality[1]. According to data from the World Health Organization (WHO), approximately 374 million new STI cases occur annually worldwide, and considering the large proportion of asymptomatic cases, the actual number is estimated to be substantially higher. In addition to causing serious health problems at the individual level, these infections also constitute a significant public health threat due to their transmission dynamics and economic burden[2].

From a urologist's standpoint, STIs are particularly important because they often present with lower urinary tract symptoms (LUTS), urethritis, prostatitis, epididymitis, and can be implicated in male infertility. Timely diagnosis is crucial to prevent long-term sequelae such as chronic prostatitis, epididymo-orchitis, and urethral stricture formation[3-5]. Young individuals are at high risk for STIs due to both biological susceptibility and a tendency toward risky sexual behaviors[3]. Factors such as inadequate sexual health education, low awareness, poor condom use, and limited access to healthcare services further increase the vulnerability of this group[4]. STIs are frequently asymptomatic, and if not diagnosed in a timely manner, they may lead to serious complications such as pelvic inflammatory disease, ectopic pregnancy, infertility, prostatitis, and neonatal infections[5,6].

Epidemiological studies conducted in Türkiye have identified *Chlamydia trachomatis*,

Neisseria gonorrhoeae, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Trichomonas vaginalis* as the most commonly encountered STI pathogens[7]. The majority of these pathogens can cause infection without manifesting clinical symptoms, thereby complicating diagnosis and treatment processes. Conventional diagnostic methods, including microscopic examination, culture, and serological tests, may be limited in terms of sensitivity and specificity, especially in asymptomatic cases with low pathogen load[8]. Furthermore, the effectiveness of these traditional methods decreases in cases of co-infection involving multiple pathogens[9]. In recent years, molecular-based diagnostic techniques have been developed to overcome these limitations, with nucleic acid amplification tests (NAATs) now widely used in clinical practice[10].

Multiplex Polymerase Chain Reaction (Multiplex PCR) offers a significant advantage in public health applications by enabling the simultaneous detection of multiple pathogens from a single clinical specimen with high sensitivity, thus providing rapid and effective diagnosis[11]. This method allows for earlier initiation of treatment, contributing to the prevention of complications and minimizing unnecessary antibiotic use, which in turn helps to reduce the emergence of antimicrobial resistance[12]. Nevertheless, despite the advances in diagnostic technology, uncertainty remains regarding the predictive value of presenting symptoms at the time of clinical admission in identifying STIs[13]. STIs often present with no symptoms or with non-specific complaints, making empirical treatment decisions difficult for clinicians. Therefore, investigating the extent to which presenting symptoms correlate with molecularly confirmed STI diagnoses emerges as an important area of inquiry that may enhance the effectiveness of clinical evaluation processes[14].

This study aims to evaluate the test results of patients presenting with urogenital symptoms to the Urology and Obstetrics-Gynecology clinics of a tertiary care university hospital who were tested for STIs using Multiplex PCR. It also investigates the correlation between presenting symptoms and molecular diagnostic outcomes, with an emphasis on the implications for urologic clinical practice.

METHODS

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Kafkas University Faculty of Medicine with the following decision date and number (03.01.2025 and 2205/01/09). 194 patients who admitted to Kafkas University, Health Research and Application Hospital, Urology and Gynecology Outpatient Clinics with sexually transmitted diseases symptoms including vaginal discharge, dysuria, pelvic pain, genital ulcers, itching and burning were enrolled in this study. The demographic data of all participants such as age, gender, and the type of symptoms were recorded. The study was conducted retrospectively.

Cervical or vaginal swab samples were taken from the canal and urethral swab samples were taken by inserting the swab 2-3 cm into the urethra and making a rotational movement. The samples were transferred to viral nucleic acid transport medium (vNAT©, Bioeksen, Istanbul, Türkiye) and transported to the Medical Microbiology Laboratory in accordance with the cold chain rules. In laboratory, nucleic acid extraction was performed by using Bio-Speedy Extraction Kit (Bioeksen, Istanbul, Türkiye) on EZ1 Zybio EXM3000 automatic nucleic acid isolation device (Bioeksen, Istanbul, Türkiye) in accordance with the manufacturer's instructions. After isolation process, Multiplex-PCR procedure was performed according to the amplification steps given in Table 1 by using "Sexually Transmitted Diseases RT-qPCR Panel"

(Bioeksen, Istanbul, Türkiye) which includes 12 different pathogens (Table 2).

Table I: Amplification steps for Sexually Transmitted Diseases RT-qPCR Panel

Steps	Cycle Count	Temperature	Duration
Reverse Transcriptase	1	52°C	3 min.
Holding	1	95°C	10 min
Denaturation	(12) Touchdown Cycle	95°C	1 sec.
Annealing/extension		67°C-56°C	15 sec.
Denaturation	30	95°C	1 sec.
Annealing/extension		95°C	15 sec.
Detection		(FAM-Green) (HEX-Yellow) (ROX-Orange) (CY5-Red)	

Table II: The microbial agents of Sexually Transmitted Diseases RT-qPCR Panel and their detection channels/wavelengths

	Pathogens	Channels	Wavelengths
1	Herpes Simplex Virus-1	FAM	520
2	*Chlamydia trachomatis*	FAM	520
3	*Treponema pallidum*	FAM	520
4	*Mycoplasma hominis*	FAM	520
5	*Mycoplasma genitalium*	HEX	550
6	*Trichomonas vaginalis*	HEX	550
7	*Haemophilus ducreyi*	HEX	550
8	*Neisseria gonorrhoeae*	ROX	610
9	*Ureaplasma parvum/urealyticum*	ROX	610
10	*Streptococcus agalactia*	ROX	610
11	Herpes Simplex Virus-2	CY5	670
12	*Gardnerella vaginalis*	CY5	670

Following PCR procedure, fluorescence signals from four different channels of the device (FAM, HEX, ROX, CY5) were obtained and analyzed. Amplification curves were evaluated using Sigmoida Analysis Software (Bioeksen, Istanbul, Türkiye). Sigmoid curves that exceeded the threshold value were recorded as "positive," while those that detected under the threshold value were recorded as "negative". In the evaluation of the results, a threshold value of 0.02 was set. After all procedures were completed, statistical analysis of the obtained data was performed using SPSS version 22.0 (IBM Corp., NY, USA) software. The Pearson Chi-square test was used to analyze categorical variables, and the One-way ANOVA test was employed to analyze continuous variables. The distribution of age was assessed using the Kolmogorov-Smirnov test, and the frequency of symptoms was analyzed through descriptive frequency analysis. Statistical significance was accepted at $p<0.05$.

RESULTS

A total of 194 participants were enrolled in this study. Of those, 142 were male (73.2%) and 52 was female (26.8%). The mean age of all participants was 43±7.2 ranging between 18 and 68. There was no statistically significant difference between age and gender (p=0.53). In our study, the symptoms of participants were separated 8 different subcategories as seen in Table 3. Some patients had more than one symptom. The most frequently observed symptom in PCR positive patients was the lower urinary tract symptoms (n=107), while the less seen was sexual function complaints (n=14).

Table III: Distribution of clinical symptoms and findings among participants

Symptom	Clinical Findings	Present (n/%)	Absent (n/%)
Lower Urinary Tract Symptom	- Dysuria - Pollakiuria - Nocturia - Burning Sensation in Urination - Post-Void Residual Sensation - Occasional Dribbling Urination - Suprapubic Pain	107 / 55.2	87 / 44.8
Urethral or Vaginal Discharge	- Urethral Discharge= Yellow, Green, Whitish, Watery, Foul Smelling - Vaginal Discharge= White, Yellow, Green Itchy, Foul Smelling	87 / 44.8	107 / 55.2
Testicular and Groin Pain	- Testicular Pain (Right Left Bilateral): Acute/Chronic, Colic Style, Pain on Palpation - Groin Pain (Right Left Bilateral: Radiating Pain in the Waist and Legs - Perineal Pain	54 / 27.8	140 / 72.2
Suspected sexual intercourse	Suspected sexual intercourse	47 / 24.2	147 / 75.8
Infertility (Pregnancy) Menstrual Irregularities and Gynecological Complaints	- Pregnancy Consideration or Planning - Vaginal Discharge During Pregnancy - Infertility-Related Complaints (Varicocele, Reduced Sperm Motility) - Mirena/Copper IUD-Associated Complaints: Bleeding, Discharge - Menstrual Irregularities - Postmenopausal Bleeding - Dysmenorrhea and Diarrhea - Bleeding or Pain During Sexual Intercourse	27 / 13.9	167 / 86.1
Systemic Infections	- Weakness - Fever - Dry Mouth - Fatigue	22 / 11.3	172 / 88.7
Urogenital Lesions	- Penis Lesions: Redness, Crusting, Itching Hard and Painless - Vaginal Lesions Wound at the entrance to the vagina - Lesions in the pubic area: Warts - Scrotal Skin Lesions: Itching, Multiple Painless Lesions - Glans Penis: Redness, Dryness, Itching	18 / 9.3	176 / 90.7
Sexual Function Complaints	- Pain During Erection - The Hardening Problem - Bad Odor / Blood in Semen - Itching after ejaculation	14 / 7.2	180 / 92.8

From 194 patients, 78 (40.2%) was diagnosed with a pathogen by Sexually Transmitted Diseases RT-qPCR Panel. The distribution of detected pathogens can be seen in Table 4.

Table IV: Distribution and frequency of pathogens detected by Multiplex PCR

Pathogens	Frequency (n)	Percentage (%)
Ureaplasma parvum	21	26.94
Gardnerella vaginalis	15	19.23
Haemophilus ducreyi	10	12.82
Neisseria gonorrhoeae	9	11.54
Mycoplasma genitalium	4	5.13
Herpes simplex virus 1	4	5.13
Mycoplasma hominis	3	3.85
Herpes simplex virus 2	1	1.28
Trichomonas vaginalis	1	1.28
Chlamydia trachomatis	1	1.28
Chlamydia trachomatis + Neisseria gonorrhoeae	4	5.12
Chlamydia trachomatis + Gardnerella vaginalis	1	1.28
Trichomonas vaginalis + Gardnerella vaginalis	1	1.28
Ureaplasma parvum + Haemophilus ducreyi	1	1.28
Mycoplasma genitalium + Haemophilus ducreyi	1	1.28
Mycoplasma hominis + Gardnerella vaginalis	1	1.28
Total	**78**	**100.0**

Table V: Distribution of clinical symptoms by sex and their association with PCR positivity

Lower urinary tract symptoms			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	93 (65.5)	49 (34.5)	0.012
Female (n)	14 (26.9)	38 (73.1)	0.315
Presence of genital discharge			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	62 (43.7)	80 (56.3)	<0.001
Female (n)	25 (48.1)	27 (51.9)	0.519
Presence of pain			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	46 (32.4)	96 (67.6)	0.359
Female (n)	8 (15.4)	44 (84.6)	0.397
Suspected sexual intercourse			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	43 (30.3)	99 (69.7)	0.027
Female (n)	4 (7.7)	48 (92.3)	0.936
Infertility (Pregnancy), Menstrual Irregularities and Gynecological Complaints			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	2 (1.4)	140 (98.6)	0.618
Female (n)	25 (48.1)	27 (51.9)	0.027
Systemic complaints			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	18 (12.7)	124 (87.3)	0.245
Female (n)	4 (7.7)	48 (92.3)	0.112
Presence of lesion			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	16 (11.3)	126 (88.7)	<0.001
Female (n)	2 (3.8)	50 (96.2)	0.735
Sexual function complaints			
	Present (n/%)	**Absent (n/%)**	**p value**
Male (n)	9 (6.3)	133 (93.7)	0.468
Female (n)	5 (9.6)	47 (90.4)	0.462
Age Groups			
	Male (n)	**Female (n)**	**p value**
	0.403	0.538	0.137

The symptoms were compared individually with the positivity rate. The association between symptoms and PCR positivity showed gender-based differences in statistical significance. LUTS was more frequently observed in males than in females (93 vs. 14), and there was a significant association between LUTS and PCR positivity (p=0.012). Similarly, the presence of genital discharge was the second most frequent symptom in males compared with females (62 vs. 25), and this

symptom was also significantly associated with PCR positivity (p<0.001). Finally, in males, 'suspected sexual intercourse' and 'presence of lesion' were significantly associated with PCR positivity, whereas in females, 'infertility (pregnancy), menstrual irregularities, and gynecological complaints' were significantly associated with PCR positivity in this study. Detailed information about the statistical analysis performed between PCR positivity and symptoms is presented in Table 5.

DISCUSSION

Sexually transmitted infections (STIs) remain a major global public health concern due to their high prevalence, transmission potential, and frequently asymptomatic course[15]. For urologists, accurate diagnosis is critical to prevent long-term sequelae such as chronic urethritis, epididymitis, prostatitis, and infertility. The limited sensitivity of conventional syndromic approaches and their inability to detect multiple pathogens simultaneously have led to the development and adoption of molecular diagnostic techniques[16]. The findings of this study demonstrate that symptom-based diagnosis alone is insufficient to guide empirical therapy, while molecular diagnostics—particularly multiplex PCR—significantly enhance diagnostic accuracy.

Conventional diagnostic strategies, although still widely used in many regions, are hampered by their inability to detect multiple pathogens concurrently and their limited performance in asymptomatic cases[17]. These shortcomings not only delay appropriate treatment but also increase the risk of persistent or recurrent infections. In contrast, multiplex PCR allows for the simultaneous detection of multiple pathogens from a single specimen with high sensitivity, enabling more accurate and individualized treatment.

According to the findings of this study, the most frequently detected pathogen was *Ureaplasma parvum* (26.94%), followed by *Gardnerella vaginalis* (19.23%) and *Haemophilus ducreyi* (12.82%). These results are largely consistent with previous Turkish studies utilizing multiplex PCR. Bakır et al. *reported U. parvum/urealyticum* as the most common pathogen (29.0%) in pregnant women, while *Mycoplasma hominis* (4.6%) and *Chlamydia trachomatis* (2.3%) were less frequent[18]. Similar findings have also been reported in a study conducted in Istanbul among high-risk male patients, where *Ureaplasma parvum* and *Gardnerella vaginalis* were identified as the most prevalent pathogens, with detection rates of 18.7% and 16.8%, respectively[19]. These results are comparable to the present study, in which *U. parvum* (26.94%) and *G. vaginalis* (19.23%) likewise ranked among the most frequently isolated microorganisms. Such consistency across different populations suggests that these pathogens may represent a common etiological spectrum in sexually transmitted infections within Turkey. In contrast, global surveillance data from the CDC and WHO identify *C. trachomatis* as the most frequently reported bacterial STI, with over 2.8 million annual cases in the United States alone[20,21]. These geographic discrepancies may be attributable to differences in study populations, healthcare-seeking behavior, diagnostic methodologies, and surveillance infrastructures. A recent systematic review of the Middle East and North Africa region similarly emphasized the wide heterogeneity of STI prevalence and highlighted the paucity of reliable epidemiological data[22].

From a urological standpoint, one of the most concerning findings is the high proportion of asymptomatic carriers. The findings of this study revealed that patients often presented with LUTS and urethral discharge; however, nearly half of (41.7%) PCR-positive cases were

asymptomatic. This finding underscores the importance of screening in high-risk populations, especially sexually active young males. This observation is consistent with WHO's 2022–2030 Global Health Sector Strategy on STIs, which stresses that asymptomatic infections pose a major challenge for syndromic management and highlights the necessity of molecular screening to identify hidden reservoirs of infection. Comparable findings have been reported in China, where Yin et al. demonstrated that 43% of women with *C. trachomatis* were asymptomatic, and the sensitivity of syndromic diagnosis was only 17% in women and 1.6% in men[2,23]. In Türkiye, PCR-based studies similarly confirmed asymptomatic carriage, such as the detection of T. vaginalis in 31.8% of women who were negative by conventional methods[24]. These data underscore not only the diagnostic limitations of symptom-based approaches but also the public health implications of untreated asymptomatic carriers, including ongoing transmission and unnecessary antibiotic use contributing to antimicrobial resistance.

Co-infections were identified in 11.5% of cases in the present study, with *C. trachomatis* and *N. gonorrhoeae* being the most frequent combination (5.12%). This finding has important implications for clinical management, as co-infections complicate empirical treatment choices. The CDC 2021 treatment guidelines emphasize that C. trachomatis and *N. gonorrhoeae* should be considered together when initiating empiric therapy for urethritis[25]. Moreover, global surveillance has highlighted the rising antimicrobial resistance (AMR) of *N. gonorrhoeae*, reinforcing the need for accurate diagnostics to guide targeted combination therapy[25,26]. Screening for co-infections is also essential from a public health standpoint, as *T. vaginalis* co-infections have been shown to facilitate HIV transmission by inducing genital mucosal disruption and enhancing viral replication[13,17]. Although only one such co-infection (n=1; 1.28%) was observed in our cohort, its potential epidemiological significance should not be overlooked.

From a demographic perspective, most PCR-positive cases occurred in patients aged 18–29 years, consistent with global data showing that over half of all STI cases occur among individuals aged 15–24[20]. Several studies among Turkish university students and young males report low STI knowledge, inadequate condom use, and high rates of unprotected first intercourse, which further exacerbate infection risk[26]. These observations highlight the urgent need for targeted public health interventions in Türkiye, particularly education programs, routine screening, and stigma reduction strategies for adolescents and young adults[27].

Gender-based differences in symptomatology were also evident in this study: LUTS and urethral discharge were significantly associated with PCR positivity in males, while infertility, menstrual irregularities, and gynecological complaints were more prominent among females. These findings align with evidence that biological, behavioral, and structural factors influence STI presentation differently across sexes[8]. Importantly, structural and gender-based inequalities in Türkiye—especially in rural settings—limit women's access to diagnostic services[28]. Addressing these inequities through equity-based restructuring of STI services, as suggested by Prescott et al. in Canada, remains a pressing priority for effective STI control[29].

CONCLUSION

In conclusion, multiplex PCR represents a pivotal tool for improving the diagnosis and management of STIs in urology. Its integration into routine evaluation, particularly in symptomatic and high-risk patients, may enhance patient outcomes and reduce

inappropriate antibiotic use. Future research should prioritize broader diagnostic panels, improved accessibility, and integration into national STI control strategies.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, the retrospective design limited control over data quality and completeness, as information was extracted from pre-existing medical records rather than prospectively collected. Second, the relatively small sample size may have reduced statistical power and restricted the generalizability of the results to broader populations. Third, the diagnostic panel was limited to 12 sexually transmitted pathogens. Other clinically significant infections such as HIV, HBV, and HCV were not included, which may have led to underestimation of the overall burden of STIs.

In addition, some sociodemographic and behavioral data—such as occupation, educational level, marital status, and history of sexual exposure—were unavailable or incompletely recorded, potentially obscuring associations with infection status. Information on sexual partners was also lacking, which precluded the assessment of transmission dynamics. Finally, due to the retrospective nature of the study, treatment regimens and clinical outcomes could not be evaluated, limiting the ability to draw conclusions regarding therapeutic efficacy.

Future research should therefore employ prospective, multicenter designs with larger cohorts, broader diagnostic panels, and detailed sociodemographic and behavioral data collection. Such studies would provide a more comprehensive understanding of STI epidemiology, transmission patterns, and treatment outcomes in the Turkish population.

Ethical approval: This study was approved by the Non-Interventional Clinical Research Ethics Committee of Kafkas University Faculty of Medicine with the following decision date and number (03.01.2025 and 2205/01/09).

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