

Article

Prevalence of Bacterial Sexually Transmitted Infections and Their Coinfections Among MSM in Mexico City

Morales-Paczka Ivonne Michele ¹, Naranjo-Bravo Jaquelin ¹, Escalera-López Melanie Alicia ¹,
García-Ángeles José de Jesús ¹, Parra-Ortega Berenice ^{1,2}, Contreras-Rodríguez Araceli ², Rivera-González Gibrán ³,
José Félix Aguirre-Garrido ⁴ and Ma. Guadalupe Aguilera-Arreola ^{1,*}

¹ Laboratorio de Bacteriología Médica, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México 11340, Mexico; imoralesp1000@alumno.ipn.mx (M.-P.I.M.); jnaranjob@ipn.mx (N.-B.J.); mescaleral1600@alumno.ipn.mx (E.-L.M.A.); jgarciaa2100@alumno.ipn.mx (G.-Á.J.d.J.); bparrao@ipn.mx (P.-O.B.)

² Laboratorio de Microbiología General, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México 11340, Mexico; acontrerasr@ipn.mx

³ Sección de Estudios de Posgrado e Investigación, Unidad Profesional Interdisciplinaria de Ingeniería y Ciencias Sociales y Administrativas, Instituto Politécnico Nacional, Ciudad de México 08400, Mexico; griverag@ipn.mx

⁴ Departamento de Ciencias Ambientales, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Unidad Lerma, Lerma de Villada, Estado de México 52005, Mexico; j.aguirre@correo.ler.uam.mx

* Correspondence: marreoag@ipn.mx

Abstract

Sexually transmitted infections remain a major public health concern among men who have sex with men (MSM), particularly due to the high frequency of coinfections and the limitations of syndromic management. This study aimed to describe the frequency of bacterial sexually transmitted and urogenital pathogens among symptomatic MSM attending two specialized sexual health clinics in Mexico City. A prospective cross-sectional study was conducted with 150 adult MSM presenting symptoms suggestive of urethritis. Urethral swab samples were analyzed using a multiplex real-time polymerase chain reaction (PCR) assay for the detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, and *Trichomonas vaginalis*. At least one pathogen was detected in 91/150 participants (60.7%). *N. gonorrhoeae* was the most frequent pathogen (33.3%), followed by *U. urealyticum* (15.3%), *M. genitalium* (9.3%), and *C. trachomatis* (9.3%). Coinfections were identified in 15 participants, representing 10.0% of the total population and 16.5% of those with a positive PCR result. Most coinfections involved *N. gonorrhoeae*, particularly with *C. trachomatis*. These findings provide descriptive microbiological evidence on the frequency and coinfection patterns of bacterial sexually transmitted infections (STIs) associated pathogens among symptomatic MSM attending specialized sexual health clinics in Mexico City. The high detection rate and occurrence of concurrent infections highlight the limitations of syndromic management and support the value of timely multiplex molecular diagnostics to improve etiological characterization, antimicrobial stewardship, and clinical decision-making in this population.

Keywords: sexually transmitted infections; men who have sex with men; multiplex PCR; coinfection



Academic Editor: Niels
Frimodt-Møller

Received: 23 May 2026

Revised: 25 June 2026

Accepted: 28 June 2026

Published: 6 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

Sexually transmitted infections (STIs) remain a major global public health concern due to their high incidence, associated complications, and their role in facilitating Human Immunodeficiency Virus (HIV) acquisition and transmission [1,2]. Men who have sex with men (MSM) represent a key population disproportionately affected by STIs, exhibiting higher infection rates driven by behavioral, biological, and structural determinants such as dense sexual networks, exposure at multiple anatomical sites, and inconsistent use of barrier protection [3,4].

Beyond their immediate clinical manifestations, STIs are associated with significant long-term complications that affect sexual and reproductive health. Untreated infections caused by pathogens such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* may lead to infertility, chronic inflammation, and damage to the reproductive tract. In men, these infections are commonly associated with urethritis and epididymal involvement, potentially compromising reproductive function [5]. In addition, persistent genital inflammation and coinfections may increase susceptibility to HIV acquisition and transmission, further amplifying their public health impact [6]. The high proportion of asymptomatic infections further exacerbates this burden, as undiagnosed individuals may unknowingly sustain transmission chains within sexual networks.

A defining characteristic of STIs in MSM is the frequent occurrence of coinfections involving multiple pathogens, together with a substantial proportion of asymptomatic cases [4]. From a microbiological perspective, the detection of these infections remains challenging due to the limitations associated with isolating and culturing several sexually transmitted microorganisms. Fastidious pathogens such as *N. gonorrhoeae* require strict growth conditions and are highly sensitive to environmental changes, which may compromise their recovery from clinical specimens. Likewise, organisms such as *Mycoplasma genitalium* and *Ureaplasma* spp. exhibit slow growth rates and demanding nutritional requirements, making their cultivation labor-intensive and often impractical in routine clinical laboratories [7]. In addition, polymicrobial communities in urogenital samples may further complicate the identification and differentiation of etiological agents.

In many clinical settings, STI management in MSM still relies on syndromic approaches, which may fail to identify asymptomatic infections and coinfections, potentially leading to inadequate treatment and persistent transmission. Advances in molecular diagnostics, particularly nucleic acid amplification tests (NAATs), have significantly improved the detection of STIs by enabling sensitive and specific identification of pathogen genetic material directly from clinical specimens [8]. Among these methods, multiplex real-time polymerase chain reaction (PCR) assays allow the simultaneous detection of multiple sexually transmitted microorganisms in a single reaction, overcoming key microbiological limitations associated with culture-based and single-target diagnostic approaches. These assays improve the detection of fastidious organisms and provide a more comprehensive characterization of coinfections, generating robust microbiological data to support clinical decision-making [9,10]. Recent studies using multiplex molecular approaches in MSM populations have demonstrated high positivity rates and frequent coinfections, reinforcing the polymicrobial nature of STIs in this key population [10,11]. Furthermore, recent epidemiological evidence highlights the persistence of a high burden of STIs and antimicrobial resistance in MSM populations, emphasizing the need for improved diagnostic strategies and surveillance systems [12].

In Mexico, available studies have documented a substantial burden of bacterial STIs among MSM and other key populations. In Tijuana, a study that included MSM and transgender women reported a high prevalence of syphilis, *C. trachomatis*, and *N. gonorrhoeae* infections, particularly among individuals newly diagnosed with HIV; importantly, most

rectal and pharyngeal CT/NG infections were asymptomatic, and many occurred in the absence of urethral infection [13]. More recently, a study conducted in Mexico City among asymptomatic MSM living with HIV reported that 26.1% of participants had at least one STI detected by urethral multiplex PCR, with *U. urealyticum*, *M. hominis*, *M. genitalium*, and *C. trachomatis* numbering among the most frequently identified microorganisms [14].

Although these studies provide important local evidence, integrated microbiological data describing bacterial STI-associated pathogens and coinfection patterns detected by multiplex molecular approaches among symptomatic MSM attending specialized sexual health clinics in Mexico remain limited. In parallel, the growing concern regarding antimicrobial resistance in *N. gonorrhoeae* in Latin America and the Caribbean underscores the need for diagnostic strategies that support etiological confirmation, targeted antimicrobial management, and linkage to culture-based antimicrobial susceptibility testing when required [7,15]. Therefore, this study aimed to describe the frequency and coinfection patterns of bacterial pathogens associated with sexually transmitted infections and detected by a commercial multiplex nucleic acid amplification test in urethral samples from symptomatic MSM attending specialized sexual health clinics in Mexico City. By applying an established molecular assay, this study provides descriptive microbiological evidence that contributes to improved characterization of the etiological profile of bacterial STIs and their coinfections in this clinical population.

2. Materials and Methods

2.1. Ethical Considerations and Approval

This study was reviewed and approved by the Research Ethics Committee of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (ENCB-IPN), with endorsement from the National Bioethics Commission (CONBIOÉTICA), under reference number SH-003-2024. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and applicable national regulations for research involving human participants. Written informed consent was obtained from all participants before sample collection. The use of clinical samples and associated data for research purposes was authorized by the institutional ethics committee. All patient information was anonymized before analysis to ensure confidentiality and data protection, and no personal identifiers were included in the study database.

2.2. Study Design and Population

This was an observational cross-sectional study conducted among men who have sex with men attending specialized sexual health clinics in Mexico City. Participants were recruited according to clinical indication for STI testing, eligibility criteria, and availability of urethral samples for molecular analysis. Therefore, the study sample should be interpreted as a clinical population and not as a representative sample of the overall MSM population in Mexico City.

This study was conducted among men who have sex with men (MSM) attending two specialized sexual health clinics in Mexico City, Mexico: Clínica Especializada Condesa (CEC) and Clínica Especializada Condesa Iztapalapa (CECI). Participants were recruited during routine clinical care between September 2024 and December 2025. Eligible participants were adult MSM presenting with symptoms suggestive of urethritis or having reported engaging in recent high-risk insertive practices and referred for STI molecular testing. Only participants with available urethral swab specimens suitable for molecular analysis were included in this study.

2.3. Urethral Sample Collection and Follow-Up

Urethral swab specimens were collected by trained healthcare personnel according to standardized clinical procedures. Samples were obtained using sterile calcium alginate swabs (Puritan Medical Products, Pittsfield, ME, United States of America (USA)) and immediately placed in sucrose–phosphate transport medium (2SP), prepared in-house using monobasic potassium phosphate (Reactivos y Productos Químicos Finos, Ecatepec de Morelos, Mexico), dibasic potassium phosphate (Sigma-Aldrich, Taufkirchen, Germany), and sucrose (Becton Dickinson, Mexico City, Mexico).

The 2SP medium was used to preserve nucleic acid integrity during transport and storage. Specimens were transported to the laboratory within 6 h of collection, maintained at 2–8 °C during transport, and stored at −20 °C until nucleic acid extraction and molecular analysis. All samples were processed under standardized pre-analytical conditions intended to preserve nucleic acid integrity and minimize DNA degradation. Samples with insufficient material or inadequate processing conditions were excluded from analysis.

Participants with a positive result for *N. gonorrhoeae* were informed of their diagnosis by the attending physicians and were advised to undergo a test of cure (TOC) at least two weeks after completing antimicrobial treatment, according to routine clinical follow-up practices.

2.4. Molecular Detection of Sexually Transmitted Pathogens

Total metagenomic DNA was extracted from urethral swab specimens using the SEEP-REP32™ automated nucleic acid extraction system (Seegene, Seoul, Republic of Korea), according to the manufacturer's instructions.

Sexually transmitted pathogens were detected using the Allplex™ STI Essential Assay (Seegene Inc., Seoul, Republic of Korea), an in vitro diagnostic (IVD)-certified multiplex nucleic acid amplification test (NAAT). This assay enables the simultaneous detection of *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum*, and *T. vaginalis*. Molecular detection was performed using the Allplex™ STI Essential Assay according to the manufacturer's instructions. This assay is a commercial multiplex real-time PCR-based NAAT designed for the simultaneous detection of major STI-associated pathogens, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, and *Trichomonas vaginalis*. The assay was used as a diagnostic molecular tool and was not developed or analytically validated as part of this study.

Amplification and real-time detection were performed using a CFX96™ Dx Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Assay setup and sample information were managed using Seegene Connect software V1.00.001 (Seegene Inc., Seoul, Republic of Korea). Amplification and data acquisition were performed using CFX Manager Dx software V 3.1.3090.102 (Bio-Rad, Pleasanton, CA, USA). Results were interpreted using Seegene Viewer IVD software V3.33.000 (Seegene Inc., Seoul, Republic of Korea), according to the manufacturer's predefined cycle threshold (Ct) criteria.

Internal, positive, and negative controls were included in each run to monitor nucleic acid extraction efficiency, assay performance, and potential PCR inhibition according to the manufacturer's instructions.

2.5. Data Collection and Statistical Analysis

Demographic, epidemiological, behavioral, clinical, and laboratory data were obtained from medical records and systematically recorded in a study database. The variables analyzed included age, age at sexual debut, number of sexual partners, substance use, sexual practices, condom or barrier use, HIV status, transactional sex, reported symptoms, and molecular detection results.

The frequencies of individual infections and coinfections were calculated for the overall study population. Coinfection was defined as the detection of two or more sexually transmitted pathogens in the same urethral specimen. The results are expressed as absolute numbers and relative frequencies. Data were captured and analyzed using Microsoft Excel V16.89 (Microsoft Corp., Redmond, WA, USA). No inferential statistical analyses were performed because of the descriptive design of the study.

3. Results

3.1. Study Population

A total of 150 men who have sex with men (MSM) were included in the study. The participants ranged in age from 19 to 53 years, and the largest age group was 25–34 years (63/150, 42.0%), followed by the 35–44 years age group (50/150, 33.3%) (Table 1). Most participants reported sexual debut between 14 and 17 years of age (77/150, 51.3%). The most common number of sexual partners per month was 2–5 (60/150, 40.0%). Overall, 85/150 participants (56.7%) were living with HIV, and transactional sex was identified in seven participants (4.7%). Substance use during sexual intercourse was documented in 115/150 participants (76.7%), and alcohol consumption was the most frequent substance use pattern (113/150, 75.3%) (Table 1).

Table 1. Sociodemographic, behavioral, and clinical characteristics of the study population.

Variable	<i>n</i> (%)
Age group (years)	
18–24	28 (18.7%)
25–34	63 (42.0%)
35–44	50 (33.3%)
≥45	9 (6.0%)
Age at sexual debut (years)	
≤13	20 (13.3%)
14–17	77 (51.3%)
≥18	53 (35.3%)
Number of sexual partners per month	
1	52 (34.7%)
2–5	60 (40.0%)
6–10	25 (16.6%)
>10	13 (8.7%)
HIV status	
Living with HIV	85 (56.7%)
Not living with HIV	61 (40.7%)
No answers provided	4 (2.6%)
Transactional sex	
Reported transactional sex	7 (4.7%)
No transactional sex reported	143 (95.3%)
No answers provided	0 (0.0%)

Table 1. *Cont.*

Variable	<i>n</i> (%)
Substance use *	
Tobacco smoking	61 (40.7%)
Alcohol consumption	113 (75.3%)
Marijuana use	57 (38.0%)
Inhaled drug use	41 (27.3%)
Injectable drug use	8 (5.3%)
Substance use during sexual intercourse	115 (76.7%)

Data are presented as absolute frequencies and percentages. Percentages were calculated using the total number of participants as the denominator ($n = 150$). * Substance use categories were not mutually exclusive; therefore, participants could report use of more than one type of substance.

3.2. Sexual Practices and Reported Symptoms

Most participants reported both insertive and receptive anal and oral sexual practices (Table 2). Both insertive and receptive anal practices were identified in 109/150 participants (72.7%), whereas both insertive and receptive fellatio was reported by 122/150 participants (81.3%). Both insertive and receptive anilingus was documented in 89/150 participants (59.3%).

Table 2. Sexual practices and reported symptoms among the study population.

Variable	<i>n</i> (%)
Sexual practices	
Insertive anal practices	33 (22.0%)
Receptive anal practices	5 (3.3%)
Both insertive and receptive anal practices	109 (72.7%)
Insertive fellatio	17 (11.3%)
Receptive fellatio	9 (6.0%)
Both insertive and receptive fellatio	122 (81.3%)
Insertive anilingus	14 (9.3%)
Receptive anilingus	20 (13.3%)
Both insertive and receptive anilingus	89 (59.3%)
Condom or barrier use	
During the last sexual encounter	40 (26.7%)
During anal sex	61/147 (41.5%)
During fellatio	10/148 (6.8%)
Latex barrier use during anilingus	1/123 (0.8%)
Reported symptoms	
Urethral discharge	88 (58.7%)
Dysuria	97 (64.7%)
Genital pruritus	45 (30.0%)

Condom use during the last sexual encounter was reported by 40/150 participants (26.7%). Condom use during anal sex was identified in 61/147 participants (41.5%), whereas condom use during fellatio was documented in only 10/148 participants (6.8%). The use of latex barriers during anilingus was uncommon (1/123, 0.8%).

The most frequent clinical manifestations were dysuria (97/150, 64.7%), urethral discharge (88/150, 58.7%), and genital pruritus (45/150, 30.0%) (Table 2).

Unless otherwise indicated, percentages were calculated using the total number of participants as the denominator ($n = 150$). For condom or barrier use during specific

sexual practices, percentages were calculated using the number of participants reporting the corresponding practice as the denominator.

3.3. Detection of Urethral Sexually Transmitted Pathogens by Multiplex PCR

At least one sexually transmitted or urogenital pathogen was detected in urethral samples from 91/150 participants (60.7%). *N. gonorrhoeae* was the most frequently detected bacterium, identified in 50/150 participants (33.3%), followed by *U. urealyticum* in 23/150 (15.3%). *M. genitalium* and *C. trachomatis* were each detected in 14/150 participants (9.3%), whereas *M. hominis* and *U. parvum* were identified in 6/150 (4.0%) and 1/150 (0.7%) participants, respectively. Although *T. vaginalis* was included in the multiplex PCR panel, no positive cases were detected in this study population.

Coinfections involving two or more pathogens were identified in 15/150 participants (10.0%), corresponding to 16.5% of participants with at least one positive PCR result. Most coinfections involved *N. gonorrhoeae* (10/15, 66.7%). The most frequent coinfection pattern was *N. gonorrhoeae* plus *C. trachomatis* (5/15, 33.3%), followed by *U. urealyticum* plus *M. hominis* (4/15, 26.7%) (Table 3). One participant presented a coinfection involving four pathogens: *N. gonorrhoeae*, *U. urealyticum*, *C. trachomatis*, and *M. hominis*.

Table 3. Coinfection patterns among sexually transmitted and urogenital pathogens detected using multiplex PCR.

Coinfection Pattern	n (%)
<i>N. gonorrhoeae</i> + <i>C. trachomatis</i>	5 (33.3%)
<i>N. gonorrhoeae</i> + <i>U. urealyticum</i>	2 (13.3%)
<i>N. gonorrhoeae</i> + <i>M. hominis</i>	1 (6.7%)
<i>N. gonorrhoeae</i> + <i>M. genitalium</i>	1 (6.7%)
<i>U. urealyticum</i> + <i>M. hominis</i>	4 (26.7%)
<i>U. urealyticum</i> + <i>M. genitalium</i>	1 (6.7%)
<i>N. gonorrhoeae</i> + <i>U. urealyticum</i> + <i>C. trachomatis</i> + <i>M. hominis</i>	1 (6.7%)
Total participants with coinfection	15 (100.0%)

Percentages were calculated using the total number of participants with coinfection as the denominator ($n = 15$).

3.4. Test of Cure

Molecular diagnostic reports were communicated to the attending physicians to support clinical management and treatment decisions. Participants with a positive result for *N. gonorrhoeae* were advised to undergo a test of cure (TOC) at least two weeks after completing antimicrobial treatment according to routine clinical follow-up practices.

Among the 50 participants positive for *N. gonorrhoeae*, only 12 returned for TOC evaluation after treatment completion. Two participants remained PCR-positive during follow-up. One participant reported subsequent sexual risk exposure after treatment, whereas the second did not return for additional follow-up after retreatment. Due to incomplete follow-up, treatment outcomes could not be fully assessed.

4. Discussion

The sociodemographic and behavioral profile of our cohort provides an important context for interpreting the observed STI burden. Most participants were young adults, mainly between 25 and 44 years of age, and more than half were living with HIV (56.7%), a proportion that reinforces the overlap between STI and HIV care in specialized sexual health settings. Frequent sexual exposure indicators were also identified, including two or more sexual partners per month in 65.3% of participants, low condom use during the last sexual encounter (26.7%), and substance use during sexual intercourse (76.7%) (Table 1). Although comparisons across studies are limited by differences in definitions and time windows,

these findings are consistent with previous reports linking multiple partners, condomless sex, and sexualized substance use with increased STI acquisition among MSM [16,17].

Reported sexual practices also suggest that exposure was not limited to the urethral site. Most participants reported both insertive and receptive anal practices (72.7%), fellatio (81.3%), and anilingus (59.3%), whereas condom or barrier use was particularly low during fellatio (6.8%) and anilingus (0.8%) (Table 2). These practices are relevant because saliva-mediated exposure has been proposed as a potential contributor to gonorrhea and chlamydia transmission to extragenital sites among MSM [18]. Because only urethral samples were analyzed, rectal and oropharyngeal infections were not detected. This is particularly relevant considering previous findings from Mexico showing that a large proportion of CT/NG infections among MSM and transgender women occurred extragenitally in the absence of urethral infection and that most rectal and pharyngeal infections were asymptomatic [13]. Thus, the urethral positivity observed in this study should be interpreted as a conservative estimate of the overall STI burden.

This study provides updated microbiological data on bacterial STI-associated pathogens detected among symptomatic MSM seeking specialized sexual health care in Mexico City. At least one pathogen was detected in a high proportion of urethral samples, supporting the relevance of multiplex NAAT-based strategies in symptomatic MSM populations. These findings highlight the value of comprehensive molecular diagnostics for identifying bacterial STI-associated pathogens and coinfections and support the need for timely and, when available, same-day molecular testing approaches to improve antimicrobial stewardship. This is particularly important in clinical settings where syndromic management or single-pathogen testing may underestimate the diversity of circulating microorganisms and fail to identify coinfections requiring different therapeutic approaches [9,10,19].

Our findings should also be interpreted considering recent studies from Mexico. In a Mexico City cohort of asymptomatic MSM living with HIV, urethral multiplex PCR detected at least one STI in 26.1% of participants, with *U. urealyticum*, *M. hominis*, *M. genitalium*, and *C. trachomatis* being the most frequent microorganisms [14]. In contrast, the higher positivity observed in our study and the predominance of *N. gonorrhoeae* are likely explained by our recruitment of symptomatic MSM with clinical suspicion of urethritis. These differences emphasize how clinical presentation, HIV status, anatomical sampling strategy, and diagnostic approach can influence the observed microbiological profile. Similarly, findings from MSM and transgender women in Tijuana showed a high burden of syphilis, *C. trachomatis*, and *N. gonorrhoeae*, particularly among individuals newly diagnosed with HIV, supporting the relevance of targeted STI testing in key populations in Mexico [13].

The overall frequency of positive results observed in this study (60.7%) is consistent with the high frequency of detected bacterial urogenital microorganisms reported in MSM populations from other geographic regions, although comparisons should be interpreted cautiously because of differences in study design, inclusion criteria, anatomical sampling sites, and diagnostic approaches. Studies from Portugal, Poland, and Taiwan have similarly reported substantial detection rates of bacterial STIs among MSM, particularly when molecular methods are used [20–22]. In our study, the elevated positivity rate was likely influenced by the inclusion of symptomatic participants with clinical suspicion of urethritis, a population enriched for urethral infections. Nevertheless, these findings indicate a considerable burden of STI-associated pathogens among MSM attending specialized sexual health services in Mexico City.

N. gonorrhoeae was the most frequently detected pathogen, both as a single infection and as part of coinfection patterns. Its frequency was higher than that reported in MSM cohorts from Lisbon, Portugal, and Gdańsk, Poland, where gonococcal infection frequencies

of 16.6% and 18.9%, respectively, have been described [20,21]. In contrast, other studies have reported a predominance of *C. trachomatis* or a more balanced distribution between *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium* [21,22]. The predominance of *N. gonorrhoeae* in our study is likely associated with the symptomatic urethritis-focused recruitment strategy (Table 2), given that gonococcal infection is strongly associated with urethral discharge and dysuria.

The predominance of *N. gonorrhoeae* should also be understood in the context of increasing concern regarding gonococcal antimicrobial resistance. A systematic review of the antimicrobial resistance of *N. gonorrhoeae* in Latin America and the Caribbean reported high resistance to penicillin, tetracycline, and ciprofloxacin, as well as emerging resistance to azithromycin, while resistance to extended-spectrum cephalosporins remained infrequent [15]. Therefore, although multiplex NAATs provide important advantages for etiological diagnosis and the detection of coinfections, culture remains essential for antimicrobial susceptibility testing, particularly for *N. gonorrhoeae*. Molecular testing and culture-based approaches should be interpreted as complementary strategies rather than mutually exclusive diagnostic tools.

Coinfections represented a clinically relevant finding, having been identified in 10.0% of the total study population and in 16.5% of participants with at least one positive result. Most coinfections involved *N. gonorrhoeae*, particularly in combination with *C. trachomatis* (Table 3). This association is clinically important because both pathogens require adequate antimicrobial coverage, and failure to detect concomitant chlamydial infection in patients treated for gonorrhea may result in incomplete therapy and persistent symptoms. Current gonorrhea management guidelines recommend antichlamydial treatment when *C. trachomatis* infection has not been excluded [23]. Therefore, multiplex diagnostic approaches may improve etiological confirmation and support more appropriate antimicrobial management in symptomatic MSM.

The identification of mixed infection patterns further supports the value of multiplex assays, as single-target testing may underestimate the polymicrobial nature of urethral infections in MSM. In addition, the detection of coinfections involving *U. urealyticum*, *M. hominis*, and *M. genitalium* suggests that microbiological results should be interpreted together with clinical findings, particularly because some urogenital microorganisms may represent colonization rather than definitive etiological agents. In this regard, the European STI Guidelines Editorial Board has cautioned against routine testing for *M. hominis* and *Ureaplasma* spp. in all patients, highlighting the need for careful clinical interpretation [24]. Nevertheless, the presence of these microorganisms in symptomatic patients and their participation in coinfection patterns may provide useful microbiological information when interpreted alongside clinical and epidemiological findings.

The detection of *M. genitalium* was also clinically relevant because this pathogen has been associated with non-gonococcal urethritis, persistent or recurrent symptoms, and increasing antimicrobial resistance worldwide. Although resistance testing was not performed in this study, the detection of *M. genitalium* in 9.3% of participants highlights the potential value of incorporating targeted molecular detection and, when available, resistance-guided strategies into STI diagnostic algorithms [25–27]. This may be particularly important in patients with persistent symptoms or previous antimicrobial exposure, in whom empirical treatment may be insufficient.

From a clinical microbiology perspective, these findings support the need for risk-informed, site-specific molecular screening in MSM, particularly among individuals living with HIV or reporting multiple partners, low barrier use, substance use during sex, and extragenital exposure. The frequent detection of *N. gonorrhoeae* and coinfections further highlights the limitations of syndromic management, which may fail to identify concomi-

tant pathogens requiring different antimicrobial approaches. This is especially relevant for pathogens such as *M. genitalium*, which require specific diagnostic and therapeutic considerations [19,26]. In our study, the molecular results were systematically communicated to treating physicians, reinforcing the role of the microbiology laboratory in etiological confirmation, diagnostic stewardship, and integrated STI/HIV care [19,23].

The low adherence to the test of cure (TOC) follow-up was another relevant operational finding. Among the 50 participants positive for *N. gonorrhoeae*, 12 (24.0%) returned for follow-up evaluation after treatment completion. Two of these 12 participants (16.7%) remained PCR-positive during follow-up, although reinfection could not be excluded in one case due to ongoing sexual exposure. These results emphasize the importance of strengthening post-treatment counseling, partner notification, and reinfection surveillance in populations with persistent exposure risk. They also highlight the challenges of evaluating treatment outcomes in real-world clinical settings.

Some limitations should be considered when interpreting these findings. The study population primarily represented symptomatic MSM attending specialized STI services, which may limit extrapolation of the results to the broader MSM population in Mexico. In addition, the cross-sectional design only provides a snapshot of infection frequency and coinfection patterns and does not allow for evaluation of temporal infection dynamics. Adherence to follow-up for TOC was limited, restricting assessment of treatment outcomes. Furthermore, only urethral samples were analyzed; therefore, we do not have an overview of the frequency of extragenital STIs. Finally, antimicrobial resistance markers were not evaluated, limiting characterization of resistance profiles in pathogens such as *N. gonorrhoeae* and *M. genitalium*. Despite these limitations, the use of a standardized multiplex NAAT provides valuable microbiological evidence on STI-associated pathogens and coinfection patterns among symptomatic MSM in Mexico, a population for which integrated molecular data remain limited.

Overall, the results support the integration of multiplex molecular approaches, particularly in specialized STI settings, as complementary tools for etiological confirmation, detection of coinfections, and antimicrobial stewardship. Nevertheless, culture-based methods remain necessary when antimicrobial susceptibility testing is required, especially for *N. gonorrhoeae*, reinforcing the need for combined diagnostic strategies in clinical microbiology and STI control programs.

5. Conclusions

This study provides descriptive microbiological evidence on the frequency of bacterial STI-associated pathogens and coinfection patterns detected by multiplex NAAT in symptomatic MSM attending specialized sexual health clinics in Mexico City. The findings support the clinical value of comprehensive molecular testing to improve etiological diagnosis and reveal coinfections that may be missed by syndromic management. However, broader studies including larger and more diverse MSM populations, extragenital sampling, and integration of culture-based antimicrobial susceptibility testing are needed to better inform STI control strategies in Mexico.

Author Contributions: Conceptualization, M.G.A.-A.; methodology, M.-P.I.M., N.-B.J., G.-Á.J.d.J. and E.-L.M.A.; investigation, M.-P.I.M., N.-B.J., J.F.A.-G. and E.-L.M.A.; resources, P.-O.B. and C.-R.A.; formal analysis, R.-G.G., M.-P.I.M. and N.-B.J.; data curation, R.-G.G., M.-P.I.M. and N.-B.J.; writing—original draft preparation, M.G.A.-A., M.-P.I.M., N.-B.J. and C.-R.A.; writing—review and editing, M.-P.I.M., E.-L.M.A., G.-Á.J.d.J., P.-O.B., C.-R.A., R.-G.G., J.F.A.-G., N.-B.J. and M.G.A.-A.; supervision, M.G.A.-A.; project administration, M.G.A.-A.; funding acquisition, M.G.A.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Secretaría de Educación, Ciencia, Tecnología e Innovación of Mexico City (SECTEI) through the project “Epidemiological surveillance of gonorrhea in vulnerable populations assisted at specialized clinics for the diagnosis of sexually transmitted infections in Mexico City” under project number SECTEI/027/2024. Additional institutional support was provided by the Instituto Politécnico Nacional (IPN), through the Secretaría de Investigación y Posgrado (SIP), under project number 20254921. M.-P.I.M. and G.-Á.J.d.J. received scholarship support from SECITHI, formerly CONACYT, and/or the BEIFI-IPN program. M.G.A.-A., C.-R.A., R.-G.G., and J.F.A.-G. are members of the Sistema Nacional de Investigadores (SNI) and receive support from institutional programs, including EDI and COFAA-IPN. N.-B.J. is a postdoctoral researcher supported by a SECITHI fellowship.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee accredited by CONBIOÉTICA under approval code SH-003-2024 (approval date: 4 July 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. No patients can be identified, and all the data were anonymized.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors thank the staff of the Clínica Especializada Condesa and Clínica Especializada Condesa Iztapalapa for their support during patient recruitment, sample collection, and clinical care. We also acknowledge the collaboration of the medical personnel involved in the interpretation of microbiological findings and patient management. The authors are especially grateful to all the participants for their voluntary participation in this study.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

MSM	Men who have sex with men
STI	Sexually transmitted infection
PCR	Polymerase chain reaction
NAATs	Nucleic acid amplification tests
TOC	Test of cure
IVD	In vitro diagnostics

References

1. Elendu, C.; Amaechi, D.C.; Elendu, I.D.; Elendu, T.C.; Amaechi, E.C.; Usoro, E.U.; Chima-Ogbuiyi, N.L.; Arrey Agbor, D.B.; Onwuegbule, C.J.; Afolayan, E.F.; et al. Global perspectives on the burden of sexually transmitted diseases: A narrative review. *Medicine* **2024**, *103*, e38199. [[CrossRef](#)] [[PubMed](#)]
2. Wilkerson, A.M.; Tao, J.; Chan, P.A. Sexually transmitted infections and risk of human immunodeficiency virus transmission. *Curr. HIV AIDS Rep.* **2025**, *22*, 40. [[CrossRef](#)] [[PubMed](#)]
3. Beyrer, C.; Baral, S.D.; van Griensven, F.; Goodreau, S.M.; Chariyalertsak, S.; Wirtz, A.L.; Brookmeyer, R. Global epidemiology of HIV infection in men who have sex with men. *Lancet* **2012**, *380*, 367–377. [[CrossRef](#)] [[PubMed](#)]
4. Marcus, J.L.; Bernstein, K.T.; Stephens, S.C.; Snell, A.; Kohn, R.P.; Liska, S.; Klausner, J.D. Sentinel surveillance of rectal chlamydia and gonorrhea among males—San Francisco, 2005–2008. *Sex. Transm. Dis.* **2010**, *37*, 59–61. [[CrossRef](#)] [[PubMed](#)]
5. Fode, M.; Fusco, F.; Lipshultz, L.I.; Weidner, W. Sexually transmitted disease and male infertility: A systematic review. *Eur. Urol. Focus* **2016**, *2*, 383–393. [[CrossRef](#)] [[PubMed](#)]
6. Ward, H.; Rönn, M. Contribution of sexually transmitted infections to the sexual transmission of HIV. *Curr. Opin. HIV AIDS* **2010**, *5*, 305–310. [[CrossRef](#)] [[PubMed](#)]

7. Unemo, M.; Seifert, H.S.; Hook, E.W., III; Hawkes, S.; Ndowa, F.; Dillon, J.R. Gonorrhoea. *Nat. Rev. Dis. Primers* **2019**, *5*, 79. [[CrossRef](#)] [[PubMed](#)]
8. Gaydos, C.A. Nucleic acid amplification tests for gonorrhea and chlamydia: Practice and applications. *Infect. Dis. Clin. N. Am.* **2005**, *19*, 367–386. [[CrossRef](#)] [[PubMed](#)]
9. Pereyre, S.; Caméléna, F.; Hénin, N.; Berçot, B.; Bébéar, C. Clinical performance of four multiplex real-time PCR kits detecting urogenital and sexually transmitted pathogens. *Clin. Microbiol. Infect.* **2022**, *28*, 733.e7–733.e13. [[CrossRef](#)] [[PubMed](#)]
10. Lee, T.-F.; Lin, K.-Y.; Chang, S.-Y.; Huang, Y.-T.; Hsueh, P.-R. Performance of two commercial multiplex polymerase chain reaction assays for the etiological diagnosis of sexually transmitted infections among men who have sex with men. *J. Microbiol. Immunol. Infect.* **2023**, *56*, 104–110. [[CrossRef](#)] [[PubMed](#)]
11. Shin, S. Epidemiologic characteristics of 1.4 million multiplex PCR tests for 12 urogenital and sexually transmitted infection pathogens in Korea (2021–2024). *Pathogens* **2025**, *14*, 1073. [[CrossRef](#)] [[PubMed](#)]
12. Müller, E.E.; Sibanda, M.; Mahlangu, M.P.; Venter, J.M.E.; Gumede, L.Y.E.; Valashiya, D.; Maseko, D.V.; Radebe, F.; Mathega, T.; Baloyi, P.; et al. Sexually transmitted infection prevalence and *Neisseria gonorrhoeae* antimicrobial resistance patterns in men who have sex with men with or without urethral discharge syndrome in Johannesburg, South Africa, 2024. *PLoS Glob. Public Health* **2026**, *6*, e0006256. [[CrossRef](#)] [[PubMed](#)]
13. Bristow, C.C.; Espinosa da Silva, C.; Vera, A.H.; Gonzalez-Fagoaga, J.E.; Rangel, G.; Pines, H.A. Prevalence of bacterial sexually transmitted infections and coinfection with HIV among men who have sex with men and transgender women in Tijuana, Mexico. *Int. J. STD AIDS* **2021**, *32*, 751–757. [[CrossRef](#)] [[PubMed](#)]
14. Sánchez Navarro, J.P.; Barriga Angulo, G.; Mata Marín, J.A.; Rodríguez Evaristo, M.; Padilla Noguera, P.E.; Gaytán Martínez, J.E. High prevalence of asymptomatic STIs in MSM PWH in a male HIV clinic in Mexico City. *J. Int. Assoc. Provid. AIDS Care* **2025**, *24*, 1–7. [[CrossRef](#)] [[PubMed](#)]
15. Sandoval, M.M.; Bardach, A.; Rojas-Roque, C.; Alconada, T.; Gomez, J.A.; Pinto, T.; Palermo, C.; Ciapponi, A. Antimicrobial resistance of *Neisseria gonorrhoeae* in Latin American countries: A systematic review. *J. Antimicrob. Chemother.* **2023**, *78*, 1322–1336. [[CrossRef](#)] [[PubMed](#)]
16. Jansen, K.; Steffen, G.; Potthoff, A.; Schuppe, A.-K.; Beer, D.; Jessen, H.; Scholten, S.; Spornraft-Ragaller, P.; Bremer, V.; Tiemann, C. STI in times of PrEP: High prevalence of chlamydia, gonorrhea, and mycoplasma at different anatomic sites in men who have sex with men in Germany. *BMC Infect. Dis.* **2020**, *20*, 110. [[CrossRef](#)] [[PubMed](#)]
17. Flores Anato, J.L.; Panagiotoglou, D.; Greenwald, Z.R.; Blanchette, M.; Trottier, C.; Vaziri, M.; Charest, L.; Szabo, J.; Thomas, R.; Maheu-Giroux, M. Chemsex and incidence of sexually transmitted infections among Canadian pre-exposure prophylaxis (PrEP) users in the l'Actuel PrEP Cohort (2013–2020). *Sex. Transm. Infect.* **2022**, *98*, 549–556. [[CrossRef](#)] [[PubMed](#)]
18. Chow, E.P.F.; Fairley, C.K. The role of saliva in gonorrhoea and chlamydia transmission to extragenital sites among men who have sex with men: New insights into transmission. *J. Int. AIDS Soc.* **2019**, *22*, e25354. [[CrossRef](#)] [[PubMed](#)]
19. Opota, O.; Greub, G. Syndromic approaches for sexually transmitted infections: Added value of molecular diagnosis. *Curr. Opin. HIV AIDS* **2025**, *20*, 303–309. [[CrossRef](#)] [[PubMed](#)]
20. Kadylak, D.; Czarny, J.; Nowicki, R.J.; Sokołowska-Wojdyło, M. The prevalence of sexually transmitted infections among male patients at a dermatovenereology outpatient clinic in Gdańsk, Poland: Findings from a single-center study. *J. Clin. Med.* **2024**, *13*, 3736. [[CrossRef](#)] [[PubMed](#)]
21. Minetti, C.; Rocha, M.; Duque, L.M.; Meireles, P.; Correia, C.; Cordeiro, D.; João, I.; Manita, C.; Soeiro, S.; Almeida Santos, J.; et al. Orogenital and anal infection by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and other sexually transmitted infections in men who have sex with men in Lisbon. *Int. J. STD AIDS* **2024**, *35*, 379–388. [[CrossRef](#)] [[PubMed](#)]
22. Wu, T.Y.; Lin, K.Y.; Su, L.H.; Sun, H.Y.; Huang, Y.S.; Liu, W.D.; Liu, W.C.; Chang, L.H.; Chang, S.Y.; Hung, C.C. Sexually transmitted coinfections among at-risk HIV-positive MSM: Implications for optimal preemptive treatment. *Front. Med.* **2024**, *11*, 1328589. [[CrossRef](#)] [[PubMed](#)]
23. Barbee, L.A.; St Cyr, S.B. Management of *Neisseria gonorrhoeae* in the United States: Summary of evidence from the development of the 2020 gonorrhea treatment recommendations and the 2021 Centers for Disease Control and Prevention sexually transmitted infection treatment guidelines. *Clin. Infect. Dis.* **2022**, *74*, S95–S111. [[CrossRef](#)] [[PubMed](#)]
24. Horner, P.J.; Donders, G.G.G.; Cusini, M.; Gomberg, M.; Jensen, J.S.; Unemo, M. Should we be testing for urogenital *Mycoplasma hominis*, *Ureaplasma parvum* and *Ureaplasma urealyticum* in men and women? A position statement from the European STI Guidelines Editorial Board. *J. Eur. Acad. Dermatol. Venereol.* **2018**, *32*, 1845–1851. [[CrossRef](#)] [[PubMed](#)]
25. Chua, T.P.; Vodstrcil, L.A.; Murray, G.L.; Plummer, E.L.; Jensen, J.S.; Unemo, M.; Chow, E.P.F.; Low, N.; Whiley, D.M.; Sweeney, E.L.; et al. Evolving patterns of macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*: An updated systematic review and meta-analysis. *Lancet Microbe* **2025**, *6*, 101047. [[CrossRef](#)] [[PubMed](#)]

26. Jensen, J.S.; Cusini, M.; Gomberg, M.; Moi, H.; Wilson, J.; Unemo, M. 2021 European guideline on the management of *Mycoplasma genitalium* infections. *J. Eur. Acad. Dermatol. Venereol.* **2022**, *36*, 641–650. [[CrossRef](#)] [[PubMed](#)]
27. Pereyre, S.; Laurier-Nadalié, C.; Le Roy, C.; Guiraud, J.; Dolzy, A.; Hénin, N.; Gardette, M.; Bébéar, C. Prevalence of macrolide and fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* in metropolitan and overseas France. *Sex. Transm. Infect.* **2023**, *99*, 254–260. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.