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Prevalence of Neisseria gonorrhoeae, Chlamydia trachomatis, Bacterial Vaginosis, Candida Species, and Mycoplasma genitalium Among Patients Attending a Sexually Transmitted Infection Clinic: A Cross-Sectional Study from Amritsar, Punjab, India

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Abstract: ***Background*: Sexually transmitted infections (STIs) remain a major public health problem in India, contributing substantially to reproductive tract morbidity, infertility, and enhanced HIV transmission. Reliable, laboratory-confirmed prevalence data- particularly using nucleic acid amplification tests (NAATs)- remain limited from tertiary care settings in northern India. *Aim and Objective*: To determine the prevalence of Neisseria gonorrhoeae, Chlamydia trachomatis, bacterial vaginosis, Candida species, and Mycoplasma genitalium among patients attending the STI clinic of a tertiary care hospital in Amritsar, Punjab. *Methods*: A cross-sectional study was conducted over one and a half years (July 2024 to December 2025) among 200 patients presenting with cervical, vaginal, or urethral discharge. Urogenital swabs were subjected to Gram/Giemsa staining, wet mount and KOH examination, culture, and PCR, as applicable. *Results*: Thirty of 200 patients (15%) were positive for at least one infection. Bacterial vaginosis was the commonest diagnosis (8, 4%), followed by Candida species (12, 6%; C. albicans 5, C. glabrata 3, C. krusei 2, C. parapsilosis 2), Mycoplasma genitalium (5, 2.5%), N. gonorrhoeae (3, 1.5%), and C. trachomatis (2, 1%). No coinfections were detected. N. gonorrhoeae and C. trachomatis were identified exclusively in males, whereas bacterial vaginosis and candidiasis were identified exclusively in females. *Limitation*: The small number of positive cases limited detailed subgroup analysis, and single-centre, hospital-based sampling may not reflect community prevalence. *Conclusion*: Molecular diagnostics substantially improved detection of fastidious, low-titre pathogens such as M. genitalium and C. trachomatis. Their integration into routine STI diagnostic protocols, together with attention to associated socioeconomic risk factors, is recommended to strengthen syndromic management and STI control programmes.***

Keywords: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, bacterial vaginosis, *Candida*, *Mycoplasma genitalium*, polymerase chain reaction, sexually transmitted infections

1. Introduction

Sexually transmitted infections (STIs) are a major global public health concern, with over 374 million new cases of four curable STIs reported annually worldwide.[1] In India, the reported prevalence of reproductive tract infections/STIs among reproductive-age women ranges from 6% to 30%.[2] A large proportion of these infections remain asymptomatic, facilitating ongoing transmission and increasing the risk of infertility, adverse pregnancy outcomes, and HIV acquisition.

Clinically, STIs are broadly classified as ulcerative and non-ulcerative. Ulcerative STIs, caused by *Treponema pallidum*, herpes simplex virus, and *Haemophilus ducreyi*, present with genital ulcers.[3] Non-ulcerative STIs typically manifest as urethral or vaginal discharge and are caused predominantly by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, the leading bacterial STI pathogens worldwide.[4] In addition, *Mycoplasma genitalium* has emerged as an important cause of non-gonococcal urethritis and cervicitis, while bacterial vaginosis and vulvovaginal candidiasis remain common causes of vaginal discharge that frequently coexist with, or mimic, STIs- posing a diagnostic challenge.

Given this overlap, syndromic management is widely recommended for patients presenting with genital discharge, although reliance on clinical features alone, without laboratory confirmation, can lead to both over- and under-treatment. Baseline, laboratory-confirmed data on STI prevalence in India remain limited, hindering the design of effective control strategies. The present cross-sectional study was therefore undertaken to determine the current prevalence of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, bacterial vaginosis, *Candida* species, and *Mycoplasma genitalium* among patients attending the STI clinic of a tertiary care hospital in Amritsar.

2. Materials and Methods

This cross-sectional study was conducted in the Sexually Transmitted Infection (STI) Clinic, Department of Microbiology, Government Medical College, Amritsar, over one and a half years, from 1 July 2024 to 31 December 2025, and included 200 patients. All patients presenting with cervical, vaginal, or urethral discharge to the STI clinic of Guru Nanak Dev Hospital, Amritsar, were enrolled after applying the inclusion and exclusion criteria. Ethical approval was obtained from the Institutional Ethics Committee,

Government Medical College, Amritsar, and written informed consent was obtained from all participants.
Vaginal and endocervical swabs were collected from female patients, and urethral swabs from male patients, under aseptic conditions following standard protocols. High vaginal swabs were self-collected by participants after standardised verbal instructions from the investigator.

Inclusion criteria comprised patients of either sex presenting with cervical, vaginal, or urethral discharge, with or without associated symptoms such as itching and lower abdominal pain, who provided informed consent. Patients who had received treatment for an STI within the preceding six months, pregnant women, and those unwilling to provide informed consent were excluded.

After obtaining written informed consent, a detailed clinical and personal history was recorded for all participants, and specimens were collected in a well-lit room. In males, three urogenital (urethral) swabs were collected using sterile Dacron swabs: the first for Gram/Giemsa staining, the second for culture on modified Thayer-Martin medium and chocolate agar, and the third preserved in phosphate-buffered medium for PCR. In females, four urogenital (endocervical and/or high vaginal) swabs were collected: the first for wet mount and KOH examination, the second for Gram/Giemsa staining, the third for culture, and the fourth preserved in phosphate-buffered medium for PCR. All samples were transported to the laboratory in appropriate media- Stuart's transport medium (HiMedia) for culture and viral transport medium for molecular analysis.

Data were entered and analysed using SPSS software version 20.0; results are expressed as frequencies and percentages.

3. Results

Table 1 presents the sociodemographic characteristics of the study participants. Of the 200 participants, most were female (81.5%), aged 25–29 years (27.5%), married (89%), and unemployed (61.5%). With respect to educational status, the largest proportion had completed primary school (25%), followed by middle school (20%) and those who were just literate (16%).

Table 1: Sociodemographic profile of study participants

Variable	Category	n (%)
Age group (years)	18–24	30 (15)
	25–29	55 (27.5)
	30–34	51 (25.5)
	35–39	41 (20.5)
	40–44	23 (11.5)
	Total	200 (100)
Sex	Male	37 (18.5)
	Female	163 (81.5)
	Total	200 (100)
Marital status	Married	178 (89)
	Unmarried	14 (7)
	Separated/Divorced	8 (4)
	Total	200 (100)
Occupation	Service sector	3 (1.5)
	Skilled	11 (5.5)
	Unskilled	63 (31.5)
	Unemployed	123 (61.5)
	Total	200 (100)
Educational level	Illiterate	14 (7)
	Just literate	32 (16)
	Primary school	50 (25)
	Middle school	40 (20)
	High school	30 (15)
	Intermediate	24 (12)
	Graduate or postgraduate	10 (5)
	Total	200 (100)

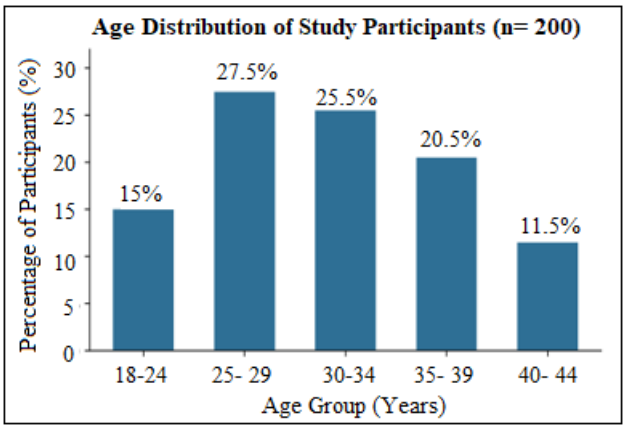

Figure 1: Age distribution of study participants (n=200)

The distribution of infections according to sociodemographic characteristics of the 30 positive individuals is presented in Table 2. *Neisseria gonorrhoeae* infections were detected exclusively in males, most commonly in the 30–34-year age group (66.6%), followed by the 35–39-year group (33.3%). *Chlamydia trachomatis* infections were also identified only in males and were equally distributed between the 25–29 and 35–39-year age groups (50% each). Bacterial vaginosis and *Candida* infections were observed exclusively in females, with the highest proportions occurring in the 18–24, 25–29, and 35–39-year groups for bacterial vaginosis (25% each) and in the 25–29-year group for *Candida* (33.3%). *Mycoplasma genitalium* infection was detected in both sexes, predominantly in males (60%), with the highest frequency in the 25–29 and 30–34-year age groups (40% each).

Most infections occurred in married participants- 100% of *N. gonorrhoeae*, 100% of *C. trachomatis*, 75% of bacterial vaginosis, 75% of *Candida*, and 80% of *M. genitalium* infections. All cases of gonorrhoea and chlamydial infection occurred among unemployed participants, whereas bacterial vaginosis was commonest among unskilled workers (62.5%), followed by unemployed individuals (37.5%). *Candida* and *M. genitalium* infections were also predominantly seen among unemployed participants (58.4% and 60%, respectively). Illiterate participants accounted for the largest proportion of *Candida* infections (33.3%), while participants with only primary education accounted for the largest proportion of gonorrhoea cases (66.7%). *Chlamydia trachomatis* infections were equally distributed between participants with primary and intermediate education (50% each), whereas *M. genitalium* infections were most frequent among those who were just literate or had intermediate education (40% each).

Table 2: Sociodemographic profile of the 30 STI-positive individuals

	N. gonorrhoeae	*C. trachomatis*	Bacterial vaginosis	*Candida spp.*	*M. genitalium*
Age (years)					
18–24	–	–	2 (25)	2 (16.6)	–
25–29	–	1 (50)	2 (25)	4 (33.3)	2 (40)
30–34	2 (66.6)	–	1 (12.5)	2 (16.6)	2 (40)
35–39	1 (33.3)	1 (50)	2 (25)	2 (16.6)	1 (20)
40–44	–	–	1 (12.5)	2 (16.6)	–
Total	3 (100)	2 (100)	8 (100)	12 (100)	5 (100)
Sex					
Male	3 (100)	2 (100)	–	–	3 (60)
Female	–	–	8 (100)	12 (100)	2 (40)
Marital status					
Married	3 (100)	2 (100)	6 (75)	9 (75)	4 (80)
Unmarried	–	–	2 (25)	2 (16.7)	1 (20)
Separated/Divorced	–	–	–	1 (8.3)	–
Occupation					
Service sector	–	–	–	–	–
Skilled	–	–	–	1 (8.3)	–
Unskilled	–	–	5 (62.5)	4 (33.3)	2 (40)
Unemployed	3 (100)	2 (100)	3 (37.5)	7 (58.4)	3 (60)
Education					
Illiterate	1 (33.3)	–	2 (25)	4 (33.3)	–
Just literate	–	–	1 (12.5)	2 (16.7)	2 (40)
Primary	2 (66.7)	1 (50)	2 (25)	–	1 (20)
Middle	–	–	1 (12.5)	1 (8.3)	–
High	–	–	1 (12.5)	1 (8.3)	–
Intermediate	–	1 (50)	1 (12.5)	2 (16.7)	2 (40)
Graduate/Postgraduate	–	–	–	2 (16.7)	–

Table 3 presents the final diagnosis distribution. Of the 200 participants, 30 (15%) were positive for at least one infection and 170 (85%) tested negative for all organisms tested. Bacterial vaginosis was the commonest diagnosis (8, 4%), followed by *Candida* species (12, 6%), *Mycoplasma genitalium* (5, 2.5%), *Neisseria gonorrhoeae* (3, 1.5%), and *Chlamydia trachomatis* (2, 1%). Among the *Candida* isolates, *C. albicans* was the commonest species (5, 41.7% of *Candida* isolates), followed by *C. glabrata* (3, 25%), and *C. krusei* and *C. parapsilosis* (2 each, 16.7%). No coinfections were detected among the 30 positive individuals.

Table 3: Final diagnosis distribution (n = 200)

Diagnosis	Positive n (%)	Negative n (%)
Neisseria gonorrhoeae	3 (1.5)	197 (98.5)
Chlamydia trachomatis	2 (1)	198 (99)
Bacterial vaginosis	8 (4)	192 (96)
Mycoplasma genitalium	5 (2.5)	195 (97.5)
Candida spp. (total)	12 (6)	188 (94)
C. albicans	5 (41.7)*	—
C. glabrata	3 (25)*	—
C. krusei	2 (16.7)*	—
C. parapsilosis	2 (16.7)*	—
Coinfection	0 (0)	—
Total	30 (15)	170 (85)

**Percentage of the 12 Candida spp. isolates.*

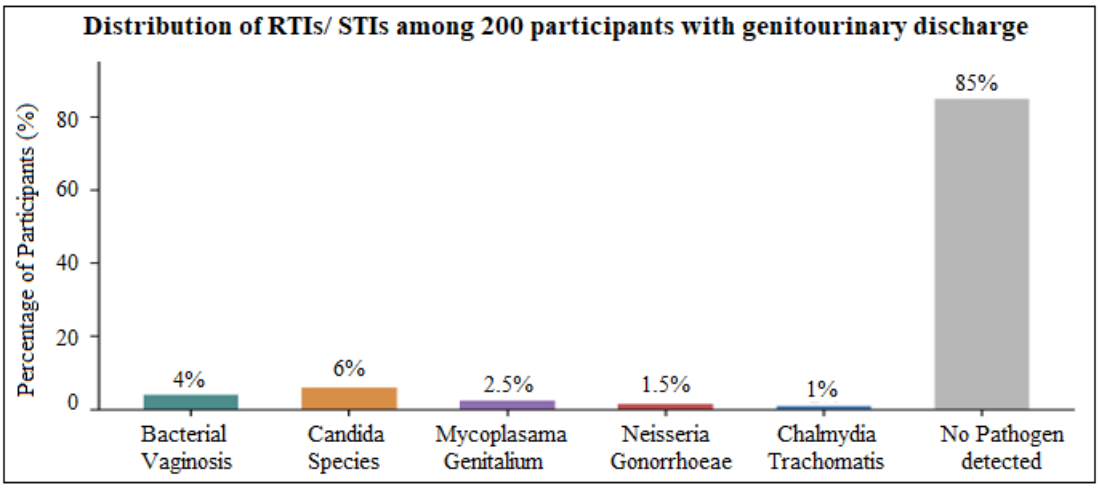

Figure 2: Distribution of RTIs/STIs among 200 participants with genitourinary discharge

For gonorrhoea, *N. gonorrhoeae* was detected in three cases (1.5%), all positive by both Gram stain and PCR; culture was negative in all three. Gram staining revealed characteristic Gram-negative, kidney-shaped intracellular diplococci. For

chlamydial infection, *C. trachomatis* was identified in two cases (1%), exclusively by PCR, with Giemsa staining yielding no positives in either case.

In bacterial vaginosis, 4 of the 8 cases (50%) were also detected on Gram stain, which demonstrated clue cells along with altered vaginal flora. All 8 patients fulfilled Amsel's criteria for bacterial vaginosis (at least three of: homogeneous vaginal discharge, vaginal pH >4.5, positive whiff test, and presence of clue cells), and all had a Nugent score of 7–10, consistent with a marked alteration of the normal vaginal microbiota.

For vulvovaginal candidiasis, all 12 cases (6%) were positive across all modalities tested- Gram stain, wet mount, KOH mount, culture on Sabouraud dextrose agar, and speciation on chromogenic agar. Microscopy demonstrated budding yeast cells, with pseudohyphae in appropriate cases; culture yielded creamy, smooth-to-pasty colonies of variable morphology, subsequently speciated on chromogenic agar. *Mycoplasma genitalium* was detected in five cases (2.5%), exclusively by PCR using SARAPLEX STI Panel (Cosara Diagnostics) reflecting the organism's fastidious growth requirements. *Mycoplasma genitalium* was detected in 5 samples with Ct values ranging from 25.26 to 34.10.

4. Discussion

Most participants belonged to the 25–34-year age group, consistent with earlier Indian studies reporting that sexually active young adults represent the population at highest risk of STIs owing to greater sexual activity and partner change.[5]

Females constituted the majority of the study population, reflecting healthcare-seeking patterns typical of facility-based studies. However, all *N. gonorrhoeae* and *C. trachomatis* infections were detected in males, likely because these infections are more often symptomatic in men. Women frequently remain asymptomatic, and endocervical infection may be missed without targeted clinical examination and sampling, potentially contributing to underdiagnosis.[6]

Although most participants were married, unmarried individuals showed a higher proportion of STI positivity, suggesting relatively greater engagement in high-risk sexual behaviour- a finding comparable to previous Indian studies.[6]

Most participants were unemployed, and all cases of gonorrhoea and chlamydial infection occurred in this group, supporting evidence that socioeconomic disadvantage, unemployment, and the associated gaps in STI awareness are linked to a higher infection burden.[7] Similarly, lower educational status was associated with higher STI positivity, with illiterate participants showing the highest prevalence- consistent with the established inverse relationship between education and STI risk, mediated through awareness and healthcare-seeking behaviour.[7]

Aravinda et al. (2022), using opa-gene PCR in a symptomatic STI-clinic population in New Delhi, reported a considerably higher gonorrhoea positivity of 14% (9% in males, 9.1% in females) and a coinfection rate of 5% between *N. gonorrhoeae* and *C. trachomatis*.[6] By contrast, the present study- which enrolled all patients presenting with discharge rather than a strictly symptomatic, syndromically defined cohort- found substantially lower positivity for both organisms (1.5% and 1%, respectively) and no coinfections. This difference likely reflects the broader inclusion criteria, the lower background prevalence in this population, and possible differences in sample size and regional STI epidemiology between the two settings, rather than a difference in assay performance; both studies used PCR-based detection, which is more sensitive than culture given the fastidious nature of *N. gonorrhoeae* and its susceptibility to adverse transport and storage conditions. Culture nonetheless remains important, as it confirms viable organisms, enables antimicrobial susceptibility testing, and complements NAATs in comprehensive diagnosis and surveillance of gonococcal infection.[6]

Chlamydia trachomatis was detected in 1% (2/200) of participants exclusively by PCR, with no positives on Giemsa staining - consistent with the recognised poor sensitivity of cytological staining for this organism. Witkin et al. (2017) reported a sensitivity below 60% for conventional staining techniques, compared with over 95% for NAATs.[8]

No coinfections were detected in the present study, in contrast to several earlier reports of frequent dual infection, particularly involving *N. gonorrhoeae* and *C. trachomatis*. This is consistent with the low overall positivity for both organisms in this cohort and supports the view- also raised by Aravinda et al.- that coinfection rates under syndromic assumptions may be considerably overestimated when compared with molecular confirmation.[6]

A key finding of the present study was that conventional methods failed to detect *Mycoplasma genitalium*, whereas PCR successfully identified *M. genitalium* in the tested samples, highlighting the superior diagnostic utility of molecular methods for detecting this fastidious pathogen. This finding is consistent with WHO guidance, which recognizes nucleic acid amplification tests (NAATs) as the preferred and most sensitive approach for detecting *M. genitalium*.[9]

5. Limitations

The small absolute number of positive cases for each organism limited the scope for detailed statistical subgroup analysis. As a single-centre, hospital-based study, the findings may not be generalisable to the wider community, and the marked female predominance in the sample may partly reflect healthcare-seeking behaviour rather than true sex-specific prevalence.

6. Conclusion

This study demonstrates the continued burden of treatable reproductive tract and sexually transmitted infections among patients attending a tertiary care STI clinic in Amritsar, with bacterial vaginosis, *Candida* species, and *Mycoplasma genitalium* emerging as the predominant diagnoses. The findings support the incorporation of nucleic acid amplification tests into routine STI diagnostic protocols, particularly for fastidious and low-titre organisms such as *C.*

trachomatis and *M. genitalium*, to enable accurate diagnosis, rational antimicrobial use, and improved patient outcomes. Addressing socioeconomic risk factors alongside expanded diagnostic capacity remains essential for reducing transmission, preventing complications, and strengthening STI control programmes.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent.

Conflicts of interest
There are no conflicts of interest.

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