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Indian Journal of Forensic and Community Medicine

Journal homepage: www.ijfcm.org

Original Research Article

Chlamydia trachomatis and genital mycoplasmas among infertility patients: A study from capital city of Uttarakhand

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Abstract

Background: Infertility, typically categorized, as primary or secondary infertility is a significant global health concern. Among the various contributing factors, sexually transmitted infections (STIs) play a major role. Notably, *Chlamydia trachomatis* and genital mycoplasmas, in addition to being common causes of STIs, are also strongly linked to infertility.

Aim: We aimed to detect the presence of *C.trachomatis* and genital mycoplasmas among the patients seeking care for infertility. The association between infection and demographic parameters was also assessed.

Materials and Methods: This cross-sectional study was conducted among the patients seeking care for infertility at the Obstetrics and Gynaecology, and Surgery Department OPD. From a total of 168 patients enrolled in the study, samples were collected which included endocervical/vaginal swabs from women and first void urine from men. Samples were subjected to semi-quantitative culture for *Ureaplasma* spp. and *Mycoplasma hominis* along with PCR assays targeting *C.trachomatis*, *Ureaplasma parvum*, *U.urealyticum*, *M.hominis* and *M.genitalium*.

Results: Among the total 168 patients, *C.trachomatis* was detected in 20.8% patients. The presence of *U.parvum*, *U.urealyticum*, *M.hominis* and *M.genitalium* was observed in 9.5%, 3.0%, 7.1% and 3.6% of patients respectively. *C.trachomatis* co-infection with *Ureaplasma* spp. and *Mycoplasma* spp. was 38.1% and 38.9% respectively. Of clinical importance, the prevalence of *C.trachomatis* and genital mycoplasma infections was significantly higher among patients over 30 years of age.

Conclusion: The detection of *C.trachomatis* and genital mycoplasmas among infertility patients highlights the need for routine screening of these pathogens as part of the standard diagnostic workup for infertility. Early and accurate diagnosis by using molecular techniques can prevent the infection transmission and associated complications including infertility.

Keywords: Adverse pregnancy outcomes, *Mycoplasma* spp., Pelvic inflammatory disease, Sexually transmitted infections, Tubal factor infertility, *Ureaplasma* spp.

Received: 12-06-2025; **Accepted:** 29-10-2025; **Available Online**: 09-12-2025

1. Introduction

Infertility, a global health problem, is defined as the inability to conceive after 12 months of regular, unprotected sexual intercourse and is usually classified as primary and secondary infertility.[1,2] Among the various factors contributing to

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https://doi.org/10.18231/j.ijfcm.11748.1761976949

human infertility, sexually transmitted infections (STIs) are recognized as one of the major and preventable causes. STIs can adversely affect fertility either directly, by damaging reproductive organs and gametes, or indirectly, by inducing inflammation that leads to tissue damage, scarring and obstruction.[3]

Globally STIs remain a major public health concern. *Chlamydia trachomatis* is currently recognized as one of the most prevalent bacterial pathogens to cause STI.[4] Furthermore, there is increasing evidence supporting the clinical significance of STIs caused by genital mycoplasmas, particularly, *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Mycoplasma genitalium*.[5,6] Beyond their role in urogenital infections, both *C.trachomatis* and genital mycoplasmas are well documented contributors to infertility.[7] An important concern associated with these pathogens is their potential to cause acute complications and long-term damage in the upper genital tract, thereby profoundly affecting reproductive health in both men and women.

Most individuals infected with *C.trachomatis* or genital mycoplasma are asymptomatic, and the infection often comes to light only during investigations for infertility. Despite the substantial evidence linking these pathogens to reproductive morbidity, routine screening for *C.trachomatis* and genital mycoplasmas has not yet become a standard component of infertility evaluation in India. In the present study, we aimed to detect the presence of *C.trachomatis* and genital mycoplasmas among the patients seeking evaluation and treatment for infertility. The association between infection and demographic parameters was also assessed.

2. Materials and Methods

2.1. Study design and Study setting

This cross-sectional, observational study was conducted in the Department of Microbiology, in collaboration with the Department of Obstetrics and Gynaecology and Department of Surgery, of a tertiary care hospital in Dehradun, Uttarakhand. Samples were also collected from Department of Obstetrics & Gynecology, of another closely located tertiary care hospital in Dehradun. The study was conducted for a period of One year (September 2023 to August 2024). The study protocol was approved by the Institutional Ethics Committee (GDMC/IEC/2023/66 and SGRR/IEC/01/24) of both the institutes and was in accordance with the declaration of Helsinki. The study protocol was explained to the participants, and prior to their enrollment, their written informed consent was obtained.

2.2. Study population

Consecutive female patients attending the infertility clinic of the aforementioned hospitals, diagnosed of primary or secondary infertility, and their male partners having normal semenogram were enrolled in the study. Women presenting with infertility were first assessed in the outpatient department through transvaginal sonography during the follicular phase along with pelvic ultrasonography, to rule out patients with polycystic ovarian syndrome, uterine fibroids, endometriosis and structural abnormalities of the reproductive tract. Baseline hormonal profiling (FSH, LH and TSH) was conducted between the 2nd and 5th day of the menstrual cycle. Except for the above, participants with evidence of genital tuberculosis, gonorrhoea and infertility attributed to male factors were also excluded from the study. Further, patients with a history of antibiotic treatment in last two months, repetitive samples from the same patients and those who were not willing to give consent were excluded.

A total of 168 patients (females: 129; Males: 39) found eligible were enrolled and underwent a thorough clinical examination conducted by an expert infertility trained gynecologist or uro-andrologist. Using a structured questionnaire, participants were interviewed to obtain information on their socio-demographic profile, risk behaviors, genital tract symptoms, prior gynaecological conditions and obstetric history.

2.3. Sample collection and processing

Three dacron-tipped endocervical swabs were collected from women and 20 ml of the first void urine samples were collected from their male partners. Of the three endocervical swabs, the first was placed in a screw capped tube containing 0.2 M sucrose phosphate buffer chlamydial transport medium for *C.trachomatis* PCR assays. Remaining two swabs were placed separately in two screw capped test tubes containing 2 ml of pleuropneumonia-like organism (PPLO) broth with urea in the first tube and arginine in the second for detection of *Ureaplasma spp*. and *M.hominis* respectively. Samples were stored at 4°C until transported to STI laboratory in the Department of Microbiology. Where the samples were subjected to semi-quantitative culture for *Ureaplasma* spp. and *M.hominis* and PCR assays for *C.trachomatis*, *Ureaplasma* spp., *M.hominis* and *M.genitalium*.

2.4. DNA extraction

DNA extraction from the samples was carried out on an automated nucleic extraction system, QIAcube Connect (Qiagen, Hilden, Germany) using QIAamp Mini Kit (Qiagen, Hilden, Germany), as per the manufacturer's instructions. The extracted DNA was stored at -20°C till further use.

2.5. Polymerase chain reaction assays

Detection of *C.trachomatis* DNA was carried out using PCR targeting a sequence within the cryptic plasmid,[8] followed by confirmation through a second PCR aimed at the *omp*A gene.[9] In addition to semi-quantitative culture, a multiplex PCR assay was performed targeting the highly conserved regions in the urease gene of *Ureaplasma spp*. and 16S rRNA of *M.hominis*, for simultaneous detection of *Ureaplasma* spp. and *M.hominis*.[10] Samples tested positive for *Ureaplasma*

spp. were further differentiated into biovar 1 (*U.parvum*) and biovar 2 (*U.urealyticum*) using an additional PCR targeting the multiple banded antigen (MBA) gene of Ureaplasma.[11] In addition, all the samples were also tested for *M.genitalium* using a PCR assay directed at the 140 kDa adhesin gene of the organism.[12] The primer sequences, target genes and the cyclic conditions used in different PCR assays are summarized in **Table 1**.

Details of the sample collection, transportation, processing for semi-quantitative culture and the detailed methodology for PCR assays is described in the supplemental file (**Supplement 1**).

2.6. Data analysis

The data was analyzed by using Statistical Package for Social Sciences (SPSS) version 28. Statistical analysis was performed using Fisher's exact test, Chi-square test and Student's t-test. $p<0.05$ was considered statistically significant.

3. Results

Of the total 168 patients enrolled in the study, 129(76.8%) were females and 39(23.2%) were male partners of the infertile women. The mean age of the women enrolled in the study was 29.93±4.37 years and the mean age of the male participants was 33.48±4.43 years. All of the patients were married.

Of the 129 female patients 63(48.8%) had primary infertility and 66(51.2%) had secondary infertility. A highly significant association was found between age and type of infertility. Of the 63 women with primary infertility, 58(92.1%) were in the <30 years age group. Of the remaining 66 women with secondary infertility 37(56.1%) were in 30-34 years age group followed by 16(24.2%) in the ≥35 years age group. Lower abdominal pain was present in 14.3%(09/63) and 48.5%(32/66) of the patients with primary and secondary infertility respectively. Tubal blockage as evidenced by hysterosalpingogram, was the predominant cause of both primary (39/63; 61.9%) and secondary (48/66; 72.7%) infertility. History of abortion and ectopic pregnancy were present respectively in 39.4%(26/66) and 18.2%(12/66) of women with secondary infertility. **Table 2** shows the association of socio-demographic and clinical characteristics with the type of infertility among the female patients.

Out of the total 168 patients, *C.trachomatis* was detected in 35(20.8%) patients (**Table 3**). Of the 39 couples enrolled in the study, 13 couples were found to be infected with *C.trachomatis*, the infection involved both partners in 06(46.1%) couples, only the male partner in 02(15.4%) couples and only the female partner in 05(38.5%) couples (**Table 4**).

Multivariate analysis was done to find the possible correlation of *C.trachomatis* with demographic and clinical characteristics of the female infertility patients and it was found that *C.trachomatis* infection were more likely to be in the age group of <30 years (48.1%). The presence of *C.trachomatis* was higher in women with secondary infertility (77.8%) than in women with primary infertility (22.2%) showing significant association between rates of chlamydial infection and type of infertility. The frequency of *C.trachomatis* infection was higher in women with tubal factor infertility (77.8%; 21/27) and was found to be a statistically significant (p=0.003) association. Infection with *C.trachomatis* was also found to be significantly associated with presence of vaginal discharge (p=0.008) and history of ectopic pregnancy (p=0.001) among the infertile women. Demographic and clinical characteristics of women with infertility in relation to *C.trachomatis* status are depicted in **Table 5**.

The overall prevalence of genital mycoplasmas among the infertility patients (n=168) was 15.5%(26/168) and 23.2%(39/168) respectively by culture and PCR. The detection rate of genital mycoplasmas in male patients was more than female patients. Of 26 culture positive samples, *Ureaplasma* spp. and *Mycoplasma* spp. were detected in 16(9.5%) and 10(6.0%) patients respectively whereas, of 39 PCR positive samples, *Ureaplasma spp.*, *M.hominis* and *M.genitalium* were detected in 21(12.5%), 12(7.1%) and 06(3.6%) patients respectively. 21 samples found positive for *Ureaplasma* spp. were further biotyped and biovar 1 and biovar 2 were detected in 16(9.5%) and 05(3.0%) samples respectively. The distribution of genital mycoplasmas among infertility patients is shown in **Table 6**.

Multivariate analysis was done to find the possible correlation of *C.trachomatis* co-infection with other STIs. The rate of co-infection of *C.trachomatis* infected patients with *Ureaplasma* spp. and *Mycoplasma* spp. was 38.1% and 38.9% respectively. One of the patient infected with *C.trachomatis* was found to have co-infection with HIV and one of the patient who tested negative for *C.trachomatis* tested positive for syphilis. **Table 7** shows the *C.trachomatis* co-infection with genital mycoplasmas, HIV and syphilis among the infertility patients.

Table 1: Details of the PCR assays used for the detection of *C. trachomatis* and genital mycoplasmas

Organism	Target gene	Primer Sequence	Cyclic conditions			Amplicon size
C. trachomatis	Cryptic plasmid	KL1 5'-TCCGGAGCGAGTTACGAAAAGA-3' KL2 5'-AATCAATGCCCGGGATTAAATTC-3'	Denaturation	94°C x 1 min	35 cycles	241 bp
			Annealing	55°C x 1 min		
			Extension	72°C x 2 min		
C. trachomatis	*omp*A gene	NLO 5'-ATGAAAAAACTCTTGAAATCG-3' NRO 5'-CTCAACTGTAACTGCGTATTT-3'	Initial denaturation	95°C x 6 min	Once	1128 bp
			Denaturation	95°C x 1 min	49 cycles	
			Annealing	45°C x 3 min		
			Extension	72°C x 3 min		
Ureaplasma spp.	Urease gene	U4 5'-ACGACGTCCATAAGCAACT-3' U5 5'-CAATCTGCTCGTGAAGTAATTAC-3	Initial denaturation	95°C x 10 min	Once	429 bp
			Denaturation	95°C x 15 secs	35 cycles	
M. hominis (Multiplex PCR)	16S rRNA gene	RNAH1 5'-CAATGGCTAATGCCGGATACGC-3' RNAH2 5'-GGTACCGTCAGTCT-3'	Annealing	60°C x 1 min		334 bp
			Extension	72°C x 5 min		
Ureaplasma spp. (PCR for Biotyping)	Multiple banded antigen gene	MBA-125 5'-GTATTTGCAATCTTTATATGTTTTCG-3' MBA-226 5'CAGCTGATGTAAGTGCAGCATTAAATTC3'	Initial denaturation	95°C x 5 min	Once	408 bp (Biovar 1) 448 bp (Biovar 2)
			Denaturation	94°C x 1 min	35 cycles	
			Annealing	56°C x 1 min		
			Extension	72°C x 1 min		
			Final extension	72°C x 5 min	Once	
M. genitalium	Adhesin gene	MgPa1 5'-AGTTGTGAAACCTTAACCCCTTGG-3' MgPa3 5'-CCGTTGAGGGGTTTTCCATTTTTGC-3'	Initial denaturation	95°C x 1 min	Once	281 bp
			Denaturation	95°C x 1 min	35 cycles	
			Annealing	67°C x 15 secs		
			Extension	72°C x 1 min		
			Final extension	72°C x 6 min	Once	

Table 2: Socio-demographic and clinical characteristics of female patients with primary and secondary infertility. (*n*=129)

Characteristics	Primary Infertility (n=63)	Secondary Infertility (n=66)	Z-test	*p-value*
Demographic characteristics				
Age in years				
• < 30	58 (92.1)	13 (19.7)	1.212	0.003*
• 30-34	01 (1.6)	37 (56.1)	3.712	0.789
• ≥35	04 (6.3)	16 (24.2)	4.001	0.042*
Education				
• Illeterate	09 (14.3)	03 (4.5)	3.213	0.089
• Primary	16 (25.4)	07 (10.6)	0.789	0.126*
• High school	35 (55.6)	28 (42.4)	2.312	0.004*
• University	03 (4.8)	28 (42.4)	1.679	0.812
Clinical characteristics				
Mucopurulent discharge				
• Yes	10 (15.9)	24 (36.4)	1.402	0.072
• No	53 (84.1)	42 (63.6)	2.193	0.012*
Lower abdominal pain				
• Yes	09 (14.3)	32 (48.5)	2.316	0.010*
• No	54 (85.7)	34 (51.5)	1.236	0.789
History ofAbortion				
• Yes	05 (7.9)	26 (39.4)	2.101	0.078
• No	58 (92.1)	40 (60.6)	1.629	0.031*
History of ectopic pregnancy				
• Yes	01 (1.6)	12 (18.2)	3.129	0.289
• No	62 (98.4)	54 (81.8)	2.105	0.017*
Causes ofinfertility				
• Tubal	39 (61.9)	48 (72.7)	1.312	0.031*
• Ovarian	16 (25.4)	05 (7.6)	2.019	0.830
• Endometriosis	05 (7.9)	08 (12.1)	0.783	0.912
• Unexplained	03 (4.8)	05 (7.6)	0.981	0.788
Menstruation				
• Regular	55 (87.3)	63 (95.5)	3.126	0.301
• Irregular	08 (12.7)	03 (4.5)	0.813	0.023*

*p<0.05 was considered statistically significant.

Table 3: *C. trachomatis* detection by in patients with Infertility. (*n*=168)

Infertility patients	Cryptic plasmid PCR and *omp*A gene PCR assay for *C. trachomatis*		Total
	Positive	Negative	
Females (n=129)	27 (20.9)	102 (79.1)	129
Males (n=39)	08 (20.5)	31 (79.5)	39
Total (n=168)	35 (20.8)	133 (79.2)	168

PCR: Polymerase chain reaction

Table 4: Couples positive for *C. trachomatis* in patients with Infertility

	Couples positive for *C. trachomatis*		
	Both partners	Females partners	Male partners
Couples with infertility (n=13)	06 (46.1)	05 (38.5)	02 (15.4%)

Table 5: Demographic and clinical characteristics of the female infertility patients with and without *C. trachomatis* infection. (*n*=129)

Characteristics	***C.trachomatis* positive (n=27)**	***C.trachomatis*negative (n=102)**	**Z-test**	***p-value***
Age in years				
• <30	13 (48.1)	58 (56.9)	3.123	**0.021***
• 30-34	09 (33.3)	29 (28.4)	4.210	0.079
• ≥35	05 (18.5)	15 (14.7)	8.153	**0.030***
Education				
• Illeterate	03 (11.1)	09 (8.8)	1.212	**0.001***
• Primary	05 (18.5)	18 (17.6)	3.1294.136	**0.030***
• High school	10 (37.0)	53 (52.0)	2.102	0.930
• University	09 (33.3)	22 (21.6)		0.103
Vaginal/ Endocervical discharge				
• Yes	23 (85.2)	11 (10.8)	1.363	**0.008***
• No	04 (14.8)	91 (89.2)	2.169	**0.013***
Abdominal Pain				
• Yes	10 (37.0)	31 (30.4)	1.303	0.713
• No	17 (63.0)	71 (69.6)	3.103	**0.003***
History of Abortion				
• Yes	06 (22.2)	25 (24.5)	1.039	0.051
• No	21 (77.8)	77 (75.5)	1.081	0.310
History of Ectopic pregnancy				
• Yes	04 (14.8)	09 (8.8)	1.013	**0.001***
• No	23 (85.2)	93 (91.2)	0.719	**0.003***
Type of infertility				
• Primary	06 (22.2)	57 (55.9)	1.813	0.130
• Secondary	21 (77.8)	45 (44.1)	2.019	0.813
Causes of Infertility				
• Tubal	21 (77.8)	66 (64.7)	3.136	**0.003***
• Ovarian	03 (11.1)	18 (17.6)	0.879	0.330
• Endometriosis	02 (7.4)	11 (10.8)	1.369	0.133
• Unexplained	01 (3.7)	07 (6.9)	0.831	0.183
Menstruation				
• Irregular	03 (11.1)	08 (7.8)	0.139	0.313
• Regular	24 (88.9)	94 (92.2)	1.393	0.513

$p<0.05$ was considered statistically significant.

Table 6: Distribution of genital mycoplasmas among the Infertility patients. (*n*=168

Infertility patients	**Culture positive**		**PCR positive**				
	Ureaplasma spp.	*Mycoplasma spp.*	*Ureaplasma spp.*	MH	UP (Biovar 1)	UU (Biovar 2)	MG
Females (n=129)	10 (7.8)	05 (3.9)	15 (11.6)	08 (6.2)	12 (9.3)	03 (2.3)	04 (3.1)
Male partners (n=39)	06 (15.4)	05 (12.8)	06 (15.4)	04 (10.3)	04 (10.3)	02 (5.1)	02 (5.1)
Total (n=168)	16 (9.5)	10 (6.0)	21 (12.5)	12 (7.1)	16 (9.5)	05 (3.0)	06 (3.6)

MG: Mycoplasma genitalium; MH: Mycoplasma hominis; PCR: Polymerase chain reaction; UP: Ureaplasma parvum; UU: Ureaplasma urealyticum

Table 7: *C. trachomatis* co-infection with genital mycoplasmas, HIV and syphilis among infertility patients. (*n*=168)

	C. trachomatis infection among Infertility patients				
Other Co-infections	Positive (%)	Negative (%)	Total	Z-test	*p-value*
Ureaplasma spp.					
• Yes	08 (38.1)	13 (61.9)	21 (100)	3.120	**0.031***
• No	27 (18.4)	120 (81.6)	147 (100)	1.796	0.414
Mycoplasma spp.					
• Yes	07 (38.9)	11 (61.1)	18 (100)	5.73	0.760
• No	28 (18.7)	122 (81.3)	150 (100)	1.77	**0.025***
Syphillis					
• Yes	0 (0.0)	01 (100)	01 (100)	0.987	0.990
• No	35 (21.0)	132 (79.0)	167 (100)		
HIV					
• Yes	01 (100)	00 (0.0)	01 (100)	0.812	0.982
• No	34 (20.4)	133 (79.6)	167 (100)	3.658	**0.004***

p<0.05 was considered statistically significant. HIV: Human immunodeficiency virus

4. Discussion

Infertility, a common and emerging health problem worldwide including India, is a cause of social, emotional, medical and economical distress for the infertile couples. STIs, particularly the ones caused by *C.trachomatis* and genital mycoplasmas are one of the major factors that contribute to the human infertility, and the association between the infection and infertility has been proven previously.[13] Majority of the infections caused by these organisms are asymptomatic, resulting in the infections to go unnoticed. Consequently, infected individuals often do not seek medical treatment and untreated, persistent, or recurrent infections may trigger a chronic immune response. This subsequently leads to enhanced production of genital immune mediators such as IL-6 and IFN- γ, which eventually can cause a considerable tissue destruction.[14] The resulting manifestations and consequences are particularly inimical to female reproductive health, substantially elevating the risk of pelvic inflammatory disease (PID) and tubal factor infertility (TFI). It is estimated that a single episode of PID increases the risk of TFI by approximately 10%. With each subsequent episode, the risk doubles, reaching nearly 40% after three or more episodes. PID although the most prevalent but is the preventable cause of TFI, provided the timely therapeutic interventions are implemented.

In the present study, *C.trachomatis* was detected in 20.8% of the patients by PCR assays. Similar detection rates of *C.trachomatis* by PCR assays have been reported in the previous studies as well.[13] We also observed that the rate of *C.trachomatis* detection was almost similar in both females (20.9%) and males (20.5%) with *C.trachomatis* detected in 13 infertile couples suggesting the need for routine screening of this organism in all infertile couples. Although earlier studies indicated that chlamydial infections predominantly affect female reproductive health and their involvement in male infertility remains controversial**,** several recent investigations have suggested otherwise.[15] It is a fact that the infected male counterpart can infect their female partners, thus further complicating the complicated. Moreover, *C.trachomatis* can interact with sperm cells via its lipopolysaccharide components, leading to the generation of reactive oxygen species that trigger sperm cell apoptosis. This process may contribute to obstructive azoospermia and impair spermatogenesis, while inflammatory cytokine production further disrupts the function of mature sperm cells.[15] Although relatively rare, ascending chlamydial infection may cause scarring and blockage of the canalicular system in the male reproductive tract, potentially impacting male fertility.

The presence of *C.trachomatis* was found to be higher among women with secondary infertility (77.8%) compared to those with primary infertility (22.2%), indicating a significant correlation between chlamydial infection and the type of infertility. A significant correlation (*p*=0.021) was also observed between *C.trachomatis* infection and age group (<30 years), indicating that women in this age group are at greater risk for infection and highlighting them as a key target population for sexual health education interventions. Although previous studies have reported that *C.trachomatis* infections are predominantly asymptomatic, especially in females, the present study, found a significant association between *C.trachomatis* infection and both vaginal discharge (*p*=0.008) and history of ectopic pregnancy (*p*=0.001) among the infertile women. A high proportion (77.8%) of *C.trachomatis* positive women were diagnosed with TFI, a statistically significant association (*p*=0.003) that aligns with findings from earlier studies. Among the various types of female infertility, TFI is most commonly linked to *C.trachomatis* infection, likely due to adnexal adhesions and tubal occlusion resulting from the infection. According to the study by Hoenderboom *et al*., *C. trachomatis* positivity represents a four-fold higher risk of TFI[16] and hence the chlamydial infections represent a silent threat to female reproductive health.

We also determined the presence of genital mycoplasmas among the patients undergoing for infertility

work-up. *Ureaplasma spp.*, *M.hominis* and *M.genitalium* were detected in 12.5%, 7.1% and 3.6% of the patients respectively. Among the 21 samples found positive for *Ureaplasma* spp. biovar 1 and biovar 2 were detected in 16(9.5%) and 05(3.0%) samples respectively. The rate of co-infection of *C.trachomatis* infected patients with *Ureaplasma* spp. and *Mycoplasma* spp. was 38.1% and 38.9% respectively. Patients infected with *C.trachomatis* are at a significantly higher risk of co-infection with other STIs, particularly genital mycoplasmas and HIV. Notably, non-ulcerative STIs caused by *C.trachomatis* and genital mycoplasmas, are known to increase the risk of both acquiring and transmitting HIV.[17] Although the present study did not specifically assess the association between genital mycoplasmas and infertility, their documented pathogenic potential underscores the importance of not overlooking their presence in infertility patients.

Genital mycoplasmas are well recognized for their detrimental effects on the reproductive health of both males and females. Where, in females, they can cause non gonococcal urethritis (NGU), bacterial vaginosis, cervicitis, endometritis, salpingitis, PID which eventually may complicate and progress to infertility,[18-21] they are also known to be associated with adverse pregnancy outcomes including premature rupture of membranes, spontaneous preterm labor, preterm birth, chorioamnionitis, spontaneous miscarriage and late abortion. In males, these organisms are known to cause NGU, balanoposthitis, prostatitis, epididymitis and orchitis.[18-21] and have been found to be associated with deteriorated semen density and pH, sperm vitality, spermatozoal motility and higher semen viscosity.[22] They can impair the function and secretions of accessory sex glands, which eventually can cause change in the seminal characterstics. The consequent inflammatory and toxic damage to the spermatogenic epithelium can lead to infertility among males as well.[18-20]

C.trachomatis and genital mycoplasmas are invariably associated with long-term complications and sequelae that can significantly impact the reproductive health of the affected individuals, hence, it is imperative to screen for these organisms, particularly among the couples seeking care for infertility. As the infections caused by these organisms fall under the preventable causes of infertility, it is also suggested that large-scale screening programs be conducted for at risk patients. Reinforcing preventive strategies by increasing awareness among people and healthcare practitioners at the primary healthcare level itself can be an efficient approach for prevention of infection transmission and associated complications.

5. Study Limitations

To the best of our knowledge, this is the first cross sectional study from Uttarakhand, to investigate the presence and association of *C.trachomatis* and genital mycoplasmas among the patients seeking care for infertility. However, our study has some limitations such as relatively smaller number of sample size, especially the men. We did not have information of the partner couple of every female patient enrolled which otherwise would have provided important information about infection concordance. Due to the resource/fund constraints, we could not perform the serovar/genovar typing for *C.trachomatis* isolates. Also for Ureaplasma isolates only the biovar typing could be done and serovar identification could not be done. Nonetheless, we believe that vital information gained from this study can serve as template for formulation of screening programs, targeted prevention and optimizing therapeutic measures, aiming to reduce the transmission of these organisms and the associated complications, particularly in terms of their association with infertility.

6. Conclusion

Infections caused by *C.trachomatis* and genital mycoplasmas pose a significant socio-economic and public health challenge. Our study findings further support the prevalence of these infections among patients with infertility. Notably, these infections were found to be more common in younger individuals, emphasizing their clinical relevance. The results highlight the need to incorporate routine microbiological screening for urogenital infections caused by *C.trachomatis* and genital mycoplasmas in the diagnostic evaluation of infertility. Importance of early and accurate diagnosis of these organisms, using highly sensitive and specific molecular techniques is also highlighted. Prompt diagnosis and targeted therapy directed towards the STIs caused by *C.trachomatis* and genital mycoplasmas are essential for effective infection management and can play a crucial role in reducing the burden of infertility associated with these organisms.

7. Source of Funding

None.

8. Conflict of Interest

None.

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Cite this article: Juyal D, Pal S, Joshi C, Kumar A, Gupta V, Rana G, Maheshwari S, Kohli S, Palariya H. *Chlamydia trachomatis* and *genital mycoplasmas* among infertility patients: A study from capital city of Uttarakhand. *Indian J Forensic Community Med*. 2025;12(4):302–310.