

# Study of gonococcal and chlamydial urethritis: Old culprits with a new story

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**Institution where the work was Conducted:** The Study was Conducted at Maulana Azad Medical College, Delhi, India

## ABSTRACT

**Introduction:** *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are the primary pathogens causing urethritis. A cross-sectional study was carried out in the Department of Microbiology in conjunction with the Department of Dermatology and STD of our hospital. The aim of the study was to detect *N. gonorrhoeae* and *C. trachomatis* among men with urethritis and to determine the anti-microbial susceptibility of the *N. gonorrhoeae* isolates. **Material and Methods:** All cases were subjected to direct Gram's smear examination and culture of urethral discharge (*N. gonorrhoeae*), real-time polymerase chain reaction and direct fluorescent antibody test (*C. trachomatis*). All *N. gonorrhoeae* isolates were subjected to anti-microbial susceptibility testing and were tested for  $\beta$ -lactamase production by chromogenic cephalosporin test. **Statistical Analysis Used:** Data were expressed as percentages. Fisher's exact test was used to evaluate statistical significance in the case of unpaired categorical data. Agreement between the methods was assessed by using kappa statistics. **Results:** Gonococcal infection was detected in 58.1% cases, and *C. trachomatis* was detected in 14% cases. However, both were detected in 12% cases. The sensitivity, specificity, positive predictive value, and negative predictive value of direct Gram's smear examination and culture of urethral discharge were found to be 100% when compared to culture for *N. gonorrhoeae*. Direct fluorescent antibody (DFA) test proved to be a valuable test aiding in the diagnosis of chlamydial urethritis with a majority of positive cases showing 20–30 elementary bodies. We detected our first gonococcal isolate with decreased susceptibility to third-generation cephalosporins, ceftriaxone, cefixime, and cefpodoxime (MIC for ceftriaxone = 0.19  $\mu$ g/ml). **Conclusions:** Optimal management of urethritis and strategies to prevent its transmission depend on accurate detection of infected persons. Our study demonstrates the utility and limitations of different laboratory tests including anti-microbial sensitivity testing for *N. gonorrhoeae* and *C. trachomatis*.

**Keywords:** Anti-microbial sensitivity testing, *Chlamydia trachomatis*, direct fluorescent antibody test, modified Thayer Martin medium, *Neisseria gonorrhoeae*, real-time PCR

## Introduction

*Neisseria gonorrhoeae* and *Chlamydia trachomatis* are primary pathogens associated with urethritis.<sup>[1]</sup> Gonorrhoea, also known as “the clap” of the 16<sup>th</sup> century, continues to be one of the most common sexually transmitted infections around the world.<sup>[2]</sup> Rising anti-microbial resistance among *N. gonorrhoeae* isolates is

a global health concern.<sup>[3]</sup> *Chlamydia trachomatis* is an important cause of non-gonococcal urethritis (NGU).<sup>[4,5]</sup> Chlamydial infections are the most commonly reported sexually transmitted infections (STIs) in the United States, with more than three times as many cases reported as gonorrhoea.<sup>[6]</sup> Recently, studies from India have reported an increase in the incidence of NGU.<sup>[7]</sup>

## Materials and Methods

A cross-sectional study was carried out between October 2011 to March 2013 in the Department of Microbiology in conjunction

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with the Department of Dermatology and STD of our hospital. Because ours is a reference laboratory for STI in North India, all adult male patients visiting our STI clinic and outpatient departments of the linked hospitals were included in the study. Study subjects were included after taking their informed consent, and patient confidentiality was maintained throughout the study.

### For detection of *N. gonorrhoeae*

Two thin sterile cotton-tipped urethral swabs were collected for Gram stain and direct inoculation onto a plate of Modified Thayer Martin medium (MTM). The plates were transported to the laboratory inside a screw-capped, wide-mouth plastic container with a candle lit inside (3–7% CO<sub>2</sub>). Growth of *N. gonorrhoeae* was suspected on the appearance of small pin-point, grey-to-white, smooth, translucent, raised convex colonies of Gram-negative cocci, oxidase-positive, superoxol-positive, and rapid carbohydrate utilisation test (RCUT), showing glucose fermentation-positive and maltose-, lactose-, and sucrose-negative. All gonococcal isolates were subjected to anti-microbial susceptibility testing by the disk diffusion method as per Clinical and Laboratory Standards guidelines (2011) using anti-microbial disks (HIMEDIA Laboratories Pvt. Ltd., Mumbai, India).<sup>[8]</sup> Control strains used were provided by Apex Regional STI Centre, Safdarjang Hospital, New Delhi. All gonococcal isolates were tested for production of β-lactamase by the chromogenic cephalosporin method (Nitrocefin disc). The minimum inhibitory concentration (MIC) of ceftriaxone for *N. gonorrhoeae* isolates was determined by the E test method using Ceftriaxone Ezy MIC™ Strip (CTR) (0.016–256 µg/ml) (HIMEDIA Catalogue Number: EM066).

### For *C. trachomatis*

The urethral swab specimen was taken for detection of *C. trachomatis* by real-time polymerase chain reaction (PCR) and direct fluorescent antibody (DFA) test according to the instructions provided in the specimen collection and transport kit, the AMPLICOR® STD swab Specimen Collection and Transport Set (Catalogue Number: 83075), and the MicroTrak® Chlamydia trachomatis Direct Specimen Test (Catalogue Number: 8H149UL), respectively.

### Case definitions

1. Gonococcal urethritis: The Gram stain of urethral smear positive for Gram-negative intracellular diplococci.
2. Non-gonococcal urethritis: An increased number of polymorphonuclear leucocytes on the Gram stain of urethral smear (≥5 PMNL/oil immersion field) negative for Gram-negative intracellular diplococci.

### Statistical analysis

Data were expressed by percentages. Fisher's exact test was used to evaluate statistical significance in the case of unpaired categorical data. Agreement between the methods was assessed by using kappa statistics. McNemar test was used to see the

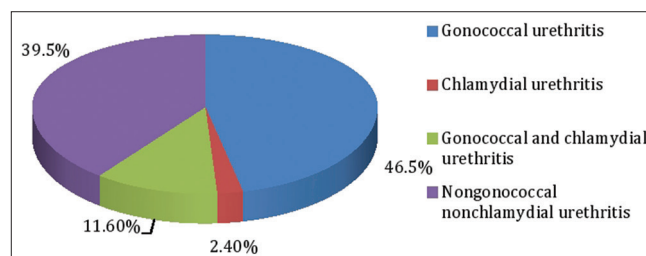
association between two methods (a *P* value less than 0.05 was considered as strong association).

## Results

Gonococcal infection was detected in 58.1% cases of urethritis, whereas *C. trachomatis* was detected in 14% cases. We found *C. trachomatis* in 5.5% cases of NGU and in 20% cases of gonococcal urethritis [Figures 1 and 2]. A majority of study subjects were between 18 and 35 years of age (86%). Twenty point nine percent subjects were illiterate. Among the study subjects, 90.7% were employed mostly in semi-skilled and unskilled labour with a monthly income between 1000 and 2000 INR. About 11.6% patients belonged to the migrant population. No significant association was found between the socio-demographic profile of the study population and aetiology of urethritis. Twenty-six (60.5%) study subjects gave the history of multiple sexual partners, and 15 (34.9%) of the cases gave the history of having indulged in paid sex. While 90.7% of study subjects gave the history of hetero-sexual contact, only 7% study subjects were regularly using barrier protection (condoms). Among all cases with the complaint of urethral discharge, 46.5% of the subjects complained of burning micturition. Two patients had inguinal lymphadenopathy, whereas one patient has genital ulcer [Table 1].

The discharge in a majority of the study subjects (51.1%) was purulent in character, moderate (53.5%) in amount, and yellowish (46.5%) in colour. Only one study subject gave the past history of urethral discharge. One subject gave the history of genital discharge in his sexual partner. Among the study population, one patient was found to be human immuno-deficiency virus-positive, whereas two study subjects were venereal disease research laboratory-reactive.

All samples that showed Gram-negative intra-cellular diplococci in direct Gram's smear examination and culture of urethral discharge were culture-positive for *N. gonorrhoeae* (sensitivity = 100%, specificity = 100%, positive predictive value = 100%, negative predictive value = 100%) [Table 2]. Following anti-microbial susceptibility testing of *N. gonorrhoeae* isolates, 96% were resistant to penicillin, whereas 84% of the isolates were ciprofloxacin-resistant. One isolate showed decreased susceptibility to ceftriaxone, cefixime, and cefpodoxime (MIC 0.19 µg/ml) [Figure 3].



**Figure 1:** Etiology of urethritis among study subjects

Out of the 43 urethral discharge specimens tested for *C. trachomatis*, six were positive by direct fluorescent antibody (DFA) test, whereas two were found to be positive by real-time PCR. The strength of agreement between real-time PCR and DFA test for detection of *C. trachomatis* was found to be good (kappa = 0.638) [Table 3].

**Table 1: Clinical symptoms reported by cases**

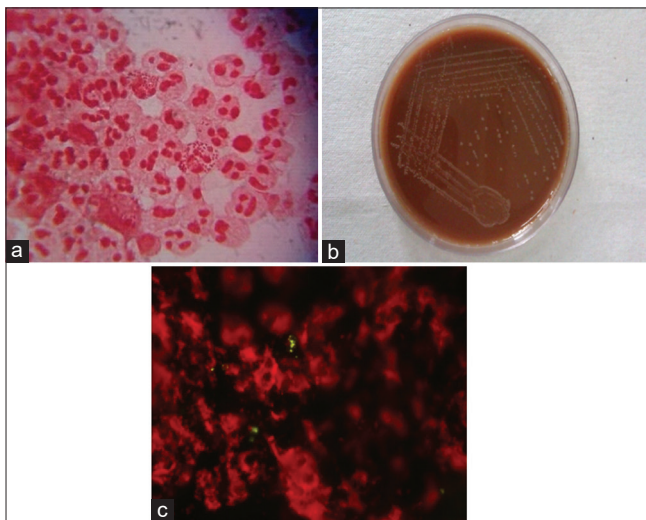
Clinical symptoms	Cases (n=43)	Percentage
Urethral discharge	43	100
Burning micturition	20	46.5
Penile swelling	3	6.9
Itching at the urethral meatus	2	4.6
Lymphadenopathy	2	4.6
Genital ulcer	1	2.3

**Table 2: Comparison between direct Gram’s smear examination and culture and culture of urethral discharge for *N. gonorrhoeae***

Intra-cellular gram-negative diplococci	Culture for <i>N. gonorrhoeae</i>		Total	P	kappa
	Positive	Negative			
Present	25	0	25	1	1
Absent	0	18	18		
Total	25	18	43		

**Table 3: Comparison between DFA test and real-time PCR for *C. trachomatis***

DFA	Real-time PCR		Total	P	kappa
	Positive	Negative			
Positive	2	4	6	0.1336	0.638
Negative	0	37	37		
Total	2	41	43		



**Figure 2:** (a) Direct Gram-stained smear of urethral discharge showing intra-cellular Gram-negative diplococci within polymorphonuclear leucocytes; (b) growth of *N. gonorrhoeae* on MTM; (c) MicroTrak® *Chlamydia trachomatis* Direct Specimen-Positive Test slide showing apple-green fluorescent elementary bodies in a background of brick-red columnar/cuboidal epithelial cells

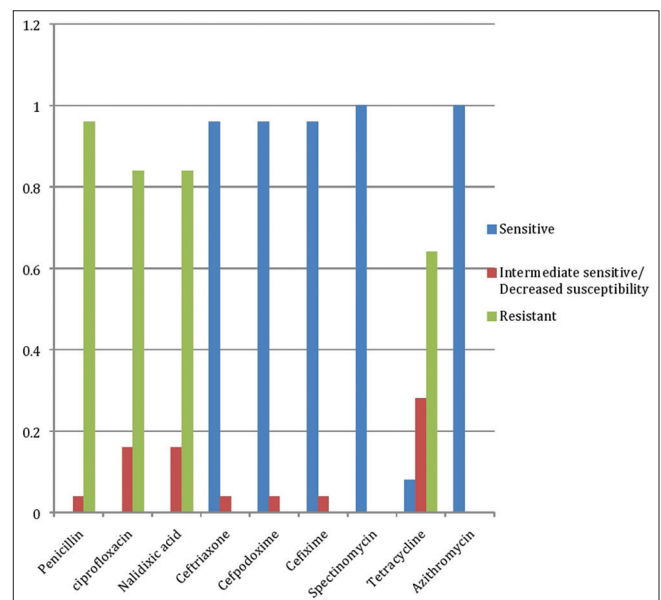
For 27 (62.7%) urethral discharge specimens, the duration between specimen collection and DNA extraction was ≤1 month. As the specimen storage period increased, a decrease in PCR positivity was noted [Table 4].

**Discussion**

In the present study, a majority (86%) of the study subjects were between 18 and 35 years of age. This is also the predominant age group for STI in other studies.<sup>[9,10]</sup> STIs are a major cause of morbidity as they affect the economically productive group. They cause a huge loss of manpower at work, measured as disability-adjusted life years (DALYs) lost.

In the present study, 90.7% of the study subjects gave the history of heterosexual contact, 60.5% of the patients gave the history of multiple sexual partners, and 34.9% gave the history of paid sex. Previous studies have reported unmarried status as a common risk factor for urethritis.<sup>[11,12]</sup> A study conducted in Assam reported 54.3% STI among married individuals, whereas extra-marital sexual relation to the extent of 68% was observed.<sup>[13]</sup> In a study conducted in GTB Hospital, Delhi, a majority of the male patients had promiscuous behaviour, with 63.9% giving the history of paid sex.<sup>[14]</sup> In our study, 7% of the cases were regularly using condoms, which was similar to a study conducted in U.K. that showed that only 8.1% patients in their study used barrier contraceptives, whereas 50% had never used any contraception.<sup>[11]</sup>

Urethral discharge and burning micturition have been reported as predominant symptoms among patients with urethritis in other studies as well.<sup>[15]</sup> In our study, all the patients presented with complaints of urethral discharge (100%), whereas burning micturition was the second-most common complaint (46.5%).



**Figure 3:** Anti-microbial susceptibility profile of the gonococcal isolates

**Table 4: Effect of duration of specimen storage prior to DNA extraction on PCR positivity for *C. trachomatis***

Specimen storage period (Months)	Number of specimens	n (%)	
		Specimens positive for <i>C. trachomatis</i> by real-time PCR	Specimens positive for <i>C. trachomatis</i> by DFA
<1	27 (62.7)	1	2
1-2	6 (14)	0	1
2-3	6 (14)	1	3
3-4	4 (9.3)	0	0
Total	43	2	6

In our study, we found direct plating of urethral swab specimens on selective media (MTM) to be a simple yet sensitive method for detection of *N. gonorrhoeae*. We found direct Gram's smear examination and culture of urethral discharge to be a rapid, inexpensive, and simple method for the diagnosis of urethritis as well as its classification into gonococcal urethritis and NGU. When compared to culture, it was found to be a highly sensitive (100%) and specific (100%) method for detection of *N. gonorrhoeae*. A positive correlation has been found between a significant number of polymorphonuclear cells in Gram-stained urethral smears and positive microbiological findings in men with urethritis in several studies.<sup>[16]</sup> The correlation between Gram staining and PCR was found to be 99.6% in a study conducted by El-Gamal *et al.*<sup>[15]</sup> However, the sensitivity of the Gram stain decreases among asymptomatic patients.<sup>[17]</sup>

Several studies have proved DFA test to be a valuable test aiding in the diagnosis of chlamydial urethritis infection.<sup>[18]</sup> It allows simultaneous assessment of specimen adequacy by visualisation of cuboidal columnar epithelial cells.<sup>[19]</sup> When compared to molecular methods, DFA test is cheap, less time-consuming, and easily available, whereas when compared to culture, it does not require maintenance of cold chains for specimen shipment and provides results faster. However, it requires a trained microscopist for interpretation of results. In our study, *C. trachomatis* was detected in six (14%) patients by DFA test, whereas it was detected in only two (4.6%) cases by real-time PCR; however, the difference between the two tests was not found to be statistically significant ( $p = 0.1336$ ). The strength of agreement between real-time PCR and DFA test was found to be good ( $\kappa = 0.638$ ). This low prevalence rate could be because of prolonged storage of samples prior to testing because of a low, erratic, and unpredictable patient turnover. It might also point towards the emergence of a new variant strain not detected by the molecular diagnostic test used by us. The sequencing of *C. trachomatis* isolated from patient specimens will be required to detect mutants that could be responsible for under-diagnosis, when relying on NAATs alone.

*Neisseria gonorrhoeae* treatment is complicated by the ability of the organism to develop resistance to a multitude of anti-microbial agents.<sup>[3,20]</sup> Surveillance projects are being conducted throughout the world [Gonococcal Isolate Surveillance Project, Gonococcal Antimicrobial Surveillance Program (GASP)] for monitoring anti-microbial resistance among *N. gonorrhoeae* isolates. Our centre

has been participating in GASP organized by the WHO-SEAR regional reference laboratory. We have witnessed a startling rise in the percentage of penicillinase-producing *N. gonorrhoeae*, quinolone-resistant *N. gonorrhoeae*, and tetracycline-resistant strains over the past 2 decades.<sup>[3]</sup> All the gonococcal isolates in our study were sensitive to ceftriaxone, cefixime, cefpodoxime, azithromycin, and spectinomycin, except one isolate, which showed decreased susceptibility to all the third-generation cephalosporins. Resistance to ceftriaxone and azithromycin is increasingly being reported all over the world.<sup>[21,22]</sup> Emergence of resistant strains is a consequence of unimpeded use and easy over the counter access of third-generation cephalosporins for several bacterial infections.<sup>[3]</sup>

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### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

### Key messages

All patients with signs and symptoms suggestive of urethritis must be screened by direct Gram's smear examination of urethral discharge. Direct plating on selective media and DFA test are highly sensitive and specific methods for detection of gonococcal and chlamydial urethritis, respectively. Anti-microbial sensitivity testing is vital in today's time.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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