

**Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in Patients Attending Central Sexually Transmitted Disease Clinic, Yangon by GeneXpert CT/NG assay**

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Sexually Transmitted Infections (STIs) are major public health problem and responsible for social, reproductive and sexual health consequences affecting men, women and children. This cross-sectional study aimed to find out *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections among 103 patients attending Central Sexually Transmitted Disease Clinic, Yangon, in 2019. Data about sociodemographic factors and associated factors was collected using structured questionnaires. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were determined from first catch urine using GeneXpert CT/NG assay. Single infection of Chlamydia, single infection of Gonorrhea and dual infection were found in 6.8%, 9.7% and 7.8%, respectively. About 42.9% of patients with single chlamydial infection, 90% with single gonococcal infection and 87.5% with dual infection were 21-30 years but its high positive rate was not statistically significant (p=0.38). Among study population, 66% were symptomatic and 34% were asymptomatic. Single chlamydial infection, single gonococcal infection and dual infection were detected in 5.9%, 13.2%, and 11.8% of symptomatic patients, respectively. Being symptomatic were significantly associated with prevalence of dual infection (p=0.03). Single chlamydial infection and single gonococcal infection were detected in 8.6% and 2.9% of asymptomatic patients, respectively. Past history of syphilis was given by 57.1%, 20% and 37.5% of patients with single chlamydial infection, single gonococcal infection and dual infection, respectively. There were statistically significant of single gonococcal infection and history of syphilis (p=0.01). This study determined positivity of asymptomatic cases of Chlamydia and Gonorrhoeae among general population. These findings highlighted need for screening of all possible STIs in high-risk persons because presence of one STI is risk factor for acquiring other STIs including HIV and syphilis. This study pointed out usefulness of GeneXpert assay in early diagnosis or screening of chlamydial and gonococcal infections especially asymptomatic cases.

*Keywords: Chlamydia trachomatis, Neisseria gonorrhoeae, STD, Urine, GeneXpert assay*

## INTRODUCTION

Sexually transmitted diseases are communicable diseases that are mainly transmitted by sexual contact and caused by bacterial, viral, Protozoa and fungal agents.<sup>1</sup> World health

Organization estimated that globally there are more than one million new cases of STDs

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every day. About 400,000 new cases of STDs occur daily in South East Asian Region.<sup>2</sup> Sexually transmitted diseases are major public health problem affecting mostly young people, in developing and developed countries.<sup>1</sup> Control of STDs represents one of aims of WHO to improve sexual health, and well-being of all people that is one of Sustainable Development Goals.<sup>2</sup>

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* are two most common sexually transmitted bacterial infections. There are annual 131 million new cases of chlamydial infection and 82 million cases of gonorrhoea worldwide. Asymptomatic reservoir of these infections provides source for disease transmission and silent disease. Untreated gonococcal and chlamydial in women result in complications such as pelvic inflammatory disease, infertility and ectopic pregnancy.<sup>1</sup> Centers for Disease Control and Prevention recommends annual chlamydia and gonorrhoea screening of sexually active women younger than 25 years, older women with risk factors such as new or multiple sex partners, or sex partner that has STI.<sup>2</sup>

There are many international studies which examined prevalence of chlamydial infections and gonorrhoea using nucleic acid amplification test on urine specimen. In a study among STD clinics attendees, Ghana, prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and co-infection were 11%, 28% and 3.2%, respectively.<sup>3</sup> A study among STD clinics attendees, Brazil reported that 13.1%, 18.4% and 4.4% were positive for chlamydial, gonorrhoea and dual infection, respectively.<sup>4</sup> A study in STD clinic attendees, Baltimore showed that prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and dual infection was 20.3%, 12.8% and 5.9%, respectively.<sup>5</sup> A study in Indonesia showed that prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and dual infection was 26.7%, 10.6% and 4.6%, respectively.<sup>6</sup>

A study conducted in STD clinics attendees, Myanmar in 2008 found that prevalence of gonococcal infection by culture and PCR

was 30.9%.<sup>7</sup> In a study among STD clinic attendees in 2010<sup>8</sup> and 2014<sup>9</sup>, prevalence of chlamydial infection was 36% and 43.08% by ICT, and gonococcal infection was 12% and 10.77% by culture. Co-infection was 1.5%.

WHO has recommended syndromic approach based on presentation of signs and symptoms. This approach can give rapid treatment and is the most realistic and cost-effective strategy for control of STDs in developing countries. Current management algorithms cannot perform for asymptomatic STDs, so etiological diagnosis with laboratory confirmation is still necessary for asymptomatic STDs.<sup>2</sup> Various specimens, including urethral, endocervical, vaginal swabs, and urine, can be utilized for diagnosis of Chlamydia and Neisseria. Collection of endocervical and urethral swab are invasive, uncomfortable and cause sociocultural implications. Compared to other collection methods, urine samples are non-invasive, easy to collect, more socially acceptable, and more useful for chlamydia and gonorrhoeae screening in routine medical care and in large population.<sup>1</sup>

Chlamydia and gonorrhoea can be diagnosed using various diagnostic techniques, including staining and microscopy with dark ground or fluorescent microscope, immunoassays and molecular methods. Nucleic acid amplification tests can use non-invasive samples, whereas others methods require invasive specimen. Nucleic acid amplification tests, including GeneXpert assay offer huge advantages in sensitivity, specificity and ease of specimen transport than other tests for diagnosis of chlamydial and gonococcal. Nucleic acid amplification tests including GeneXpert are particularly useful for detection of difficult to cultivate pathogens such as Chlamydia and Neisseria, simultaneous detection of these infections in single clinical specimen, and detection of apparent infection.<sup>1</sup> GeneXpert assays are automated nucleic acid amplification assays that using cartridge-based platform, resulting in minimal steps and contamination than conventional nucleic acid amplification tests. On testing urine samples, sensitivity of this

assay for *Chlamydia trachomatis* is 98.5% in males and 98.1% in females, and for *Neisseria gonorrhoeae* is 98.3% in males and 94.4% in females. Specificity of this assay for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* is 99.9% for both male and female.<sup>6</sup> This study aimed to determine positivity of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in patients attending Central STD clinic by using GeneXpert assay on urine.

## MATERIALS AND METHODS

A cross-sectional study was conducted in total 103 male and female patients attending Central STD Clinic, Yangon from January to October 2019. Eligible participants were patients 16 years aged and older attending Central STD Clinic. Women who were pregnant, menstruating and revisited patients who had participated in the study at previous visit were excluded. Every weekday during clinic's opening hours, patients who met eligibility criteria attending STD clinic were enrolled to participate. Minimum required sample was 103 which have been calculated by using formula  $N = \frac{4Z^2p(1-p)}{w^2}$ .

### *Study procedure*

After getting informed consent, socio-demographic characteristics and clinical data were collected using structured questionnaire. Specimens were collected from symptomatic and asymptomatic males and females attending at the Clinic. Urine collection cup was provided to patients to collect about 20 ml of urine. Urine samples were stored at 4-8°C and tested with GeneXpert assay within two hours after collecting samples. GeneXpert assay was performed for detection of DNA of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and qualitative results were obtained within 90 minutes.

### *Operational definitions*

#### Symptomatic sexually transmitted infections

A patient was classified as symptomatic if patient reported having one or more of

symptoms associated with sexually transmitted infections such as discharge from penis, vagina, sores or warts on genital area, painful or frequent urination, itching in genital area, enlarged inguinal lymphnode and pain in lower abdomen.

#### Asymptomatic sexually transmitted infections

A patient was classified as asymptomatic if neither of these clinical symptoms were reported.

### *Principle of GeneXpert assay*

GeneXpert assay is automated qualitative real time assay for simultaneous detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. GeneXpert assay detects CT1 for *Chlamydia trachomatis*, NG2 and NG4 for *Neisseria gonorrhoeae*. The assay has built in probe check control, sample processing control and sample adequacy control, which detects single copy human gene. GeneXpert assay uses single use cartridges, which enables automated sample preparation, extraction, amplification, and detection of target sequences.

### *Interpretation of results*

Results were automatically interpreted by GeneXpert instrument system and qualitatively reported as "DETECTED" or "NOT-DETECTED" for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

### *Data analysis*

Data were collected by using proforma and data entry was done by using Microsoft Office Excel 2010. Data analysis was done using Microsoft Office Excel 2010 and SPSS 16.0. Chi-square test and Fisher's Exact test were used to determine significance of associations between categorical variables. If 'p' value was less than 0.05, association was considered to be statistically significant.

### *Ethical consideration*

This study was done according to guidelines issued by Research and Ethics Committee, University of Medicine 1 and was carried out after approval of Postgraduate Academic Board of Studies. Informed consent was

taken after thorough explanation of study. Confidentiality of information collected was strictly maintained. Result was used only for health care and research purpose.

## RESULTS

Sociodemographic characteristics and associated factors are presented in Table 1. Among total 103 participants in this study, 74 (71.8%) were males and 29 (28.2%) were females. The mean and median ages of study population were 32 years and 28 years, respectively.

Table 1. Sociodemographic characteristics and associated factors of study population

Categories	Frequency	Percentage
<b>Age group (Years)</b>		
16-20	8	7.8
21-30	56	54.4
31-40	13	12.6
41-50	20	19.4
51 and above	6	5.8
<b>Gender</b>		
Male	74	71.8
Female	29	28.2
<b>Marital status</b>		
Single	46	44.7
Married	50	48.5
Widowed	2	1.9
Separated	4	3.9
Divorced	1	1
<b>Educational level</b>		
Illiterate	2	1.9
Primary school	13	12.6
Middle school	25	24.3
High school	40	38.8
Graduate	23	22.3
<b>Clinical presentations</b>		
<i>Symptomatic</i>		
Dysuria	44	64.7
Frequency	32	47.1
Discharge	25	36.8
Genital ulcer	18	26.5
Inguinal lymphadenopathy	13	19.1
Pruritus	12	17.6
<i>Asymptomatic</i>		
	35	34

Among total 103 patients, chlamydial and gonococcal infection were detected in 25 (24.3%), in which Single infection of chlamydia was seen in 7 (6.8%), Single infection of gonorrhoea in 10 (9.7%) and dual infection in 8 (7.8%) (Fig. 1).

Of the 7 cases of single chlamydial infection, 6 cases (85.7%) were male patients and a case (14.3%) was female. All 10 cases of single gonococcal infection were males. Out of 8 cases of dual infection, 7 cases (87.5%) were detected in male population and 1 case (12.5%) was female. Males were more commonly seen in all infection groups. There was no association between type of STI and patient's sex ( $p=0.06$ ).

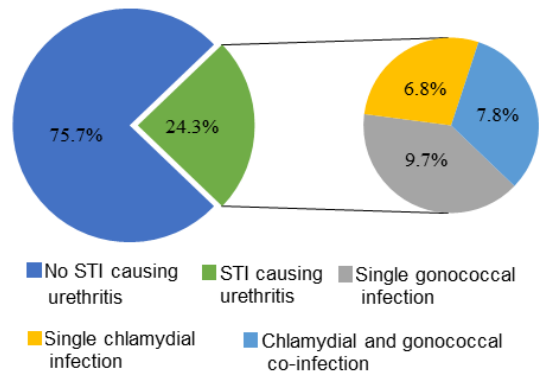


Fig. 1. Prevalence of chlamydial and gonococcal infection among patients attending Central STD clinic

Majority of single chlamydial infection, single gonococcal infection and dual infection were found in 21-30 years age group. Any association was not found between type of STI and patient's age ( $p=0.38$ ). Most of patients (66%, 68/103) were symptomatic and remaining 35 patients (34%) were asymptomatic. Of the symptomatic patients, 5.9% had single chlamydial infection, 13.2% had single gonococcal infection and 11.8% had dual infection. Being symptomatic were significantly associated with prevalence of dual infection ( $p=0.03$ ). Prevalence of single chlamydial infection and single gonococcal infection among asymptomatic patients were 8.6% and 2.9%, respectively.

Among these 68 symptomatic patients, dysuria was the most common symptom (64.7%)

Table 2. Prevalence of single chlamydial infection, single gonococcal infection and dual infection by sociodemographic characteristics and associated factors

Characteristics	Single chlamydial infection (N=7) n(%)	Single gonococcal infection (N=10) n(%)	Chlamydial and gonococcal coinfection (N=8) n(%)	'p' value
<i>Age group (Years)</i>				0.38
16-20	0(0)	1(10)	0(0)	
21-30	3(42.9)	9(90)	7(87.5)	
31-40	1(14.3)	0(0)	0(0)	
41-50	2(28.6)	0(0)	1(12.5)	
51 and above	1(14.3)	0(0)	0(0)	
<i>Gender</i>				0.06
Male	6(85.7)	10(100)	7(87.5)	
Female	1(14.3)	0(0)	1(12.5)	
<i>Marital status</i>				0.0001
Single	4(57.1)	10(100)	5(62.5)	
Married	3(42.9)	0(0)	1(12.5)	
Widowed	0(0)	0(0)	0(0)	
Separated	0(0)	0(0)	2(25)	
Divorced	0(0)	0(0)	0(0)	
<i>Educational level</i>				0.09
Illiterate	0(0)	0(0)	0(0)	
Primary school	0(0)	1(10)	1(12.5)	
Middle school	1(14.3)	1(10)	0(0)	
High school	6(85.7)	6(60)	5(62.5)	
Graduate	0(0)	2(20)	2(25)	
<i>Clinical presentations</i>				
<i>Symptomatic</i>				
Dysuria	4(5.9)	9(13.2)	8(11.8)	0.03
Frequency	3(42.9)	8(80)	7(87.5)	0.11
Discharge	2(28.6)	7(70)	6(75)	0.04
Genital-ulcer	0(0)	6(60)	5(62.5)	0.03
Inguinal lymphadenopathy	0(0)	1(10)	1(12.5)	0.25
Pruritus	0(0)	3(30)	0(0)	0.45
<i>Asymptomatic</i>	1(14.3)	0(0)	1(12.5)	0.19
<i>Past medical history of syphilis</i>	3(8.6)	1(2.9)	0(0)	
<i>Past medical history of syphilis</i>	4(57.1)	2(20)	3(37.5)	0.01

followed by frequent urination (47.1%), urethral discharge (36.8%), genital ulcer (26.5%), enlargement of inguinal lymph node (19.1%) and pruritus (17.6%). Dysuria (51.4%) was the most common presenting features in male patients, and vaginal discharge and pruritus (each 27.6%) were the most common presenting symptoms in females.

The most commonly reported symptom of patients with chlamydial and gonococcal was dysuria: 42.9% in single chlamydial infection patients, 80% in single gonococcal infection patients and 87.5% in dual infection patients. About 28.6% of single chlamydial infection patients, 70% of single gonococcal infection patients and 75% of co-infection patients reported frequent urination. Discharge was reported in 60% of single gonococcal infection patients and 62.5% of dual infection patients. Discharge (p=0.03) and frequent urination (p=0.04) were significantly associated with infection (Table 2). About 85.7% of single chlamydial infection had high school education and 14.3% had middle school. Of 10 cases of single gonococcal infection, 60% had high school level, 20% graduated and each 10% had primary and middle school. Among chlamydial and gonococcal co-infected cases, 62.5% had high school, 25% had graduated and 12.5% had primary school. No significant difference among those infection in aspect of patients' educational level (p =0.09). About 57.1% of patients with single chlamydial infection were single and 42.9% were married. All single gonococcal infection patients were single. Among chlamydial gonococcal co-infection cases, 62.5% were single, 25% were separated and 12.5% were married. Infection rates were significantly higher in unmarried participants than married (p=0.0001) (Table 2).

Past medical history of syphilis was given by 57.1%, 20% and 37.5% of patients with single chlamydial infection, single gonococcal infection and dual infection, respectively. Single gonococcal infection cases gave past history of syphilis more

frequently than single chlamydial infection and dual infection ( $p=0.01$ ) (Table 2).

## DISCUSSION

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* cause major public health consequences due to asymptomatic in nature, prevalence among adolescents and reproductive morbidity. STDs are still high prevalent in developing countries, including Myanmar, due to economic hardship, lack of STDs control programme and lack of access to STDs services. Information on epidemiological data of STDs is essential for development of successful prevention and management strategies in country.<sup>3</sup> This study provided information about prevalence of chlamydial infection and gonorrhoea in STD clinic attendees, who are high risk persons of being suspected of having exposure to STD.

The prevalence of single chlamydial infection and single gonococcal infection in this study were 6.8% and 9.7%, respectively. Present study found low prevalence rate than local previous studies conducted at same sexually transmitted disease clinic in 2010<sup>8</sup> and 2014<sup>9</sup>, which reported prevalence rate of 30% and 35.4% by Chlamydial antigen test for single chlamydial infection, and 12% and 9.2% by staining and culture for single gonococcal infection, respectively.

The prevalence rates of this study were slightly higher than that of STD clinic attendees, Barcelona<sup>10</sup> and China<sup>11</sup> that used same diagnostic test and specimen type, with prevalence rates of 4.3% and 6.3% for chlamydial infection and 4.5% and 8.4% for gonococcal infection. Prevalence of this study were lower than that in STD clinic attendees, Malaysia<sup>12</sup> and Ghana<sup>3</sup> that used same diagnostic technique and specimen type, which prevalence of chlamydial infection was 11.6% and 11%, and of gonococcal infection was 24.2% and 28%, respectively. Present study was conducted at central STD clinic only, so prevalence of this study may not reflect prevalence of

community and it may be lower than total prevalence in community.

Prevalence of chlamydial and gonococcal dual infection in this study was 7.8% that was 46.7% and 38.9% of overall prevalence of chlamydia and gonococcal infection, respectively. This co-infection rate was consistent with results of previous studies among STD clinic attendees, Malaysia<sup>12</sup> and India<sup>13</sup> which reported 8.4% and 14%, respectively. This finding reinforces importance of testing for both infections.

Approximately half of STD clinic attendees in this study were within 21-30 years and found that high prevalence of single chlamydial infection (42.9%), single gonococcal infection (90%) and co-infection (87.5%) were in this age group. This finding of study was comparable with another Myanmar study among STD clinic attendees,<sup>7</sup> which reported highest proportion of infection (86.3%) was found between 20-40 years, and study among STD clinic attendees, Malaysia,<sup>12</sup> which reported that 47.4% of attendees were between 20-29 years and highest rate of chlamydial (7/11) and gonococcal infection (11/23) was found in this group. These findings showed that majority of STD clinic attendees were young adults which are sexually active reproductive age group. There is need to grant reproductive health education relating to STIs in particular to teenagers and young adults who may have possibly less knowledge and less experience with STI prevention.

Among cases of single chlamydial infection in this study, 6 cases (85.7%) were male patients and 1 case (14.3%) was female. All cases of single gonococcal infection were males. Of the 8 cases of dual infection, 7 cases (87.5%) were detected in male and 1 case (12.5%) in female. Both chlamydial and gonococcal infections were found more in male than females, but there was no association between type of STI and patient's sex ( $p=0.06$ ). These findings were agreement with findings of other studies. A study

conducted at same place in 2014 which reported 6 cases of gonococcal infection were detected among male and 1 case among females,<sup>9</sup> and study among STD clinic attendees, Bissau which reported gonococcal infection was more prevalent in male (38.7%) than female (17%)<sup>14</sup> and among STD clinic attendees, Taiwan, chlamydial infection was more prevalent in male (66%) than female (34%).<sup>15</sup> The findings of lower rates of infection among females in this study may be due to use of urine specimen because most common site of infection for both chlamydia and gonorrhoea in women is cervix.<sup>3</sup> Another reason is small number of female participant which may be due to more likely asymptomatic nature of infection compared to male, social stigma, and more likely to confuse STD symptoms for something else. This finding suggested that STD campaigns should encourage increasing STD clinic attendance of females since they are underrepresented at STD clinics, and some strategies should incorporate into STD and reproductive health services.

In this study, 8.6% and 2.9% of asymptomatic patients had single chlamydial infection and single gonococcal infection, respectively. Single chlamydial infection, single gonococcal infection and dual infection were found in 5.9%, 13.2% and 11.8% of symptomatic patients. Presence of symptoms was found to be significantly associated with prevalence of dual infection ( $p=0.03$ ).

In Taiwan, prevalence of chlamydial infection in asymptomatic and symptomatic STD clinic attendees were 14% and 23%, respectively, and reported symptomatic patients were more likely infection positive than asymptomatic ( $p<0.05$ ),<sup>15</sup> and in Bangkok, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were detected in 3% and 16% of asymptomatic, and 22% and 6% of symptomatic patients, respectively.<sup>16</sup> No further testing of oral sample in asymptomatic patients of this study for oropharyngeal infection. Inferences from this study may be limited regarding possibility of oropharyngeal infection in asymptomatic

patients. Present study's findings regarding asymptomatic infections highlighted importance of screening these infections among high-risk persons with no symptoms for control of STI more effectively.

In this study, dysuria was commonly reported symptom among patients with single chlamydial infection (42.9%), single gonococcal infection (80%) and co-infection (87.5%) followed by frequent urination (28.6%, 70% and 75%) and discharge (60% and 62.5%). According to Situ's study<sup>12</sup> and Dela's study<sup>3</sup> among STD clinic attendees, urethral discharge was most common symptom for both chlamydial and gonorrhoea, and second most common symptom was dysuria. Discharge ( $p=0.03$ ) and frequent urination ( $p=0.04$ ) were found to be significantly associated with prevalence of infection in this study, which in accordance with previous finding in Taiwan.<sup>15</sup>

Although most frequent cause of dysuria is urinary tract infection, sexually transmitted infections like chlamydial urethritis can be presented with dysuria and resembled as urinary tract infection. Every patient with urethritis should be suspected to have gonorrhoea and chlamydial urethritis and investigations on them should contain tests for these sexually transmitted infections. About 57.1% of single chlamydial infection patients, 20% of single gonococcal infection patients and 37.5% of co-infection patients reported history of serologically diagnosed syphilis. There was statistically significant association between type of infection and past history of syphilis. Single gonococcal infection cases gave past history of syphilis more frequently than single chlamydial infection and dual infection ( $p=0.01$ ). Blair's study<sup>17</sup> and Divekar's study<sup>18</sup> found that syphilis with chlamydial and gonorrhoea co-infection in 13.8% and 18.8%, respectively. These findings reiterate importance of routine screening of all possible STIs in high-risk individuals.

### Conclusion

Findings of this study highlighted need for screening programme for apparently healthy

persons with risky behaviors to get STIs in order to break down silent transmission and control of STIs more effectively. This study suggested to use GeneXpert assay as useful diagnostic tool for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, because it requires urine samples which can be non-invasively collected and it generates results within short period of time. More studies in different epidemiological settings are needed for further evaluation of GeneXpert assay for Chlamydia and Neisseria detection, especially their cost-effectiveness, as affordability has critical implications for scaling up this technology, particularly in resource poor settings like Myanmar. Because GeneXpert assay is unable to perform antimicrobial susceptibility testing, this study is unable to generate information regarding antimicrobial susceptibility, particularly for *Neisseria gonorrhoeae*, which has developed drug resistance. Therefore, large scale study including antimicrobial susceptibilities of gonococci should be conducted in future to determine prevalence of chlamydial and gonorrhea and trend of antimicrobial susceptibility gonococci.

#### Competing interests

The authors declare that they have no competing of interests.

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