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Assessment of *Chlamydia trachomatis* prevalence and associated risk factors among men who have sex with men (MSM) in Chennai using real-time PCR: a cross-sectional study

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ABSTRACT

Background: *Chlamydia trachomatis* (CT) infection poses a significant public health concern, particularly among men who have sex with men (MSM) due to their increased risk of transmission. The asymptomatic nature of CT infections underscores the importance of routine screening initiatives. Diagnostic techniques, such as nucleic acid amplification tests (NAATs), remain the gold standard for CT detection. However, conventional PCR has limitations like false positivity and reduced specificity. Real-time PCR is a molecular diagnostic technique with high sensitivity and specificity. Hence the present study was designed to screen the anal swabs obtained from MSM for the presence of CT using real-time PCR.

Methods: Anal swabs were obtained from the MSM population. DNA extraction was done and real-time PCR to detect outer membrane protein complex B gene was performed.

Results: Out of 103 patients screened, 4 were found to be positive for CT. One among the positive patients had co-infection with syphilis.

Conclusion: Rapid detection of CT using real-time PCR serves as an effective screening tool in establishing early diagnosis and initiating prompt therapy among MSM.

KEYWORDS: *Chlamydia trachomatis*; Real time polymerase chain reaction; Men who have sex with men; diagnosis; sexually transmitted infection

INTRODUCTION

Chlamydia trachomatis (CT) is a Gram-negative, obligate intracellular pathogen which is known to cause urethritis, proctitis and lymphogranuloma venereum [1]. Globally, 128.5 million new cases of CT are reported each year, making it the most commonly reported bacterial STI worldwide [2]. Sexual intercourse with an infected partner can occur through vaginal, anal, or oral sex. Youths between the ages of 15 and 24 are more prone to develop this infection. Since Chlamydial infections are asymptomatic, majority of those

affected are unaware of their condition and do not seek medical care. This makes it more difficult to detect these infections early and to treat them clinically, especially in developing nations [3].

Men who have sex with men (MSM) are men who have sexual activity with members of the same sex. In India, same-sex relationships and behavior are typically far more informal. Male homosexuality is associated with anal sex and oral sex or mutual masturbation. MSM avoid seeking routine or appropriate health care as they are treated unfairly by the public and medical professionals. MSM who participate in high-risk sexual behaviors pose the danger of contracting HIV and other STIs, either for themselves or their partners [4]. Studies have observed that MSM in India have several sexual partners and frequently avoid using condoms or other forms of protection [5]. In India, MSM are becoming more and more recognized as a population at risk for HIV and other STIs [6]. The Physician has to be comfortable in asking about their sexual practices routinely [7]. The swab specimen can be collected from anal, rectal and oral region in MSM patients. The swabs specimens are considered suitable for detecting asymptomatic STI patients, as they are relatively non-invasive specimens and have high sensitivity for pathogen detection than other specimens [8].

Diagnosis of *C.trachomatis* infection is done by various methods like direct cytological examination, antigen detection using enzyme immuno assays, nucleic acid detection using probes, nucleic acid amplification techniques and isolation in cell culture [9]. Out of these methods nucleic acid amplification is found to have high sensitivity. Conventional PCRs are designed to detect various genes of CT but the problem with them is contamination and lack of specificity. In recent years, real-time PCR has become more and more popular as a method of identifying genital *Chlamydia* infection because it is simpler to use, yields faster results, and, because it is conducted in a closed-tube format, is less likely to be contaminated [10]. Due to their great sensitivity and reliability when compared to culture, these tests are frequently employed in western nations [11]. However, due to their high cost, these devices are not very useful for routine diagnostics in low-resource nations like India. The application of in-house real-time PCR assays that are as sensitive and specific as those produced by commercial molecular amplification techniques will therefore present a chance for less expensive testing to identify genital *Chlamydia* infection in developing countries [12].

METHODOLOGY

Study centre:- Samples were collected from the Department of Venerology, Rajiv Gandhi Government General Hospital (RGGH), Chennai. Ethical approval for which has been already obtained from the hospital for this study.

Ethical number– 29042023

Sample collection

MSM patients were chosen and their demographic details, previous medical history, clinical history, marital status, contact (pre-marital and extramarital) history and prior treatment history were collected from the hospital records. Informed consent was obtained from all the patients enrolled in the study. Anorectal swabs were collected by the physician and transported to the laboratory in a viral transport medium for molecular diagnosis. If transport is delayed >24 hours, the transport media containing the specimen was stored at −70°C.

DNA extraction

The DNA was extracted from the sample using Qiagen DNeasy mini kit (Qiagen, Germany) as per the kit protocol. The purity of the extracted DNA samples was quantified by spectrophotometric method and the samples were stored at -4ºC for further use.

DNA Amplification by Real Time Polymerase chain reaction:

Real Time Polymerase Chain Reaction targeting the outer membrane protein complex B (Amplificon size 106bp) was performed for *C.trachomatis* detection. *C.trachomatis* DNA obtained from National Institute for Research in Reproductive Health (NIRRH) Mumbai was used as a positive control for this assay. Primer and Probe sequence were designed by Eurofins Genomics India Pvt.Ltd based on a previous publication (Butcher et.al 13). The sequence of the primers and probe used are listed in the Table 1. The Real Time polymerase chain reaction conditions used were 50 °C for 2min, 95 °C for 10min, followed by 45 cycles of 95 °C for 15s and 60 °C for 60s.

RESULT

A total of 103 MSM were recruited for this study. Demographic characteristics of the study population are given in the Table 2. The mean age of participants was 29.03 years. The chief presenting complaints of the patients were urethral discharge (4.9%), genital ulcer (3.9%), anal discharge (2%), genital warts (2%) and burning micturition (1%), Previous history of STI was found in 19.4% of the study population. The diseases that caused STI previously are depicted in figure 1. Newly diagnosed STI among the study population were HIV (14.6%) and syphilis (36%).

Out of 103 patients, 4 were positive for CT infection by Real time polymerase chain reaction. Amplification plot of Real time polymerase chain reaction done on the study samples are depicted in figure 2. The *Ct* values of the positive samples are tabulated in Table.3. One of the CT positive patients was found to have a co-infection with syphilis.

DISCUSSION

In recent years, the incidence of STI among MSM had significantly increased worldwide when compared to female sex workers and other population [14]. In our study population, majority of MSM (55%) were infected with syphilis. However, a recent study conducted by National AIDS Control Organization reported that the syphilis seropositivity in India is getting low and stable [15]. The report of the phase IV and extension period of National AIDS Control Program states that the prevalence of STI among MSM has reduced from 2.1% in 2013-2014 to 0.99% in 2019-2020. It was also observed that the condom demand has decreased among MSM over years [16].

In our study, we detected CT in 3.8% of MSM patients. This is in accordance with a previous Indian study which reported prevalence of CT infection among 4% of symptomatic patients attending STI clinic [17]. Another multi-centric Indian study conducted among MSM in Chennai and Mumbai reported a prevalence rate of 11.3% and 18.2% respectively. About 19.4% of our study population had a past history of STI which was in line with the previous study which reported 18.6% of previous STIs [18]. A similar study conducted in China among MSM reported CT in 8.0% (9/112) of the study group [19]. The men included in our study

were predominantly aged between 19-28 years (54.36%). It was observed that, 26.21% of the study population were alcoholic, 21.35% were smokers and 11.65% had tattoos. The prevalence of alcoholism in our study was much lesser when compared with the Chinese study which reported alcoholism in 77.37% of the study population. Unprotected anal intercourse was very common and was found in 72.23% of our study population in contrast to 66% reported by a previous study [19]. The sexual practices adopted by our MSM patients were oro-receptive (75.7%), oro-insertive (73.7%), ano-insertive (66%) and ano-receptive (68.9%). Anal sex was found to be more common in our study population (67.5%) when compared with the studies done in China (34.7%) and Vietnam (45.6%) [19, 20].

Among our study population, 3% were found to be commercial sex workers and 17.5%were HIV positive (3 old cases and 15 new cases). An Australian study done in MSM reported 18% commercial sex workers with 22% HIV positivity [21].

The real time PCR was able to detect very low copy number and had a higher sensitivity compared to conventional PCR amplifying cryptic plasmid & omp1 gene in a previous study [22]. The prevalence of CT using real-time PCR in our study was 3.8%which is higher than the prevalence reported by conventional PCR (2.2%) in a study conducted in Mumbai [23]

CT infection is a major public health concern among men who have sex with men (MSM), with significant implications for individual health and public health burden. Real-time PCR offers high sensitivity and specificity, enabling accurate detection of CT infections, particularly in asymptomatic individuals. Early identification of CT infections through screening, facilitates prompt treatment and reduces the risk of complications. Moreover, CT screening among MSM contributes to the prevention of secondary transmission within sexual networks and the overall reduction of CT prevalence in the community. Real-time PCR-based screening methods provide rapid results, allowing for timely initiation of treatment and partner notification efforts. Furthermore, the implementation of CT screening programs using real-time PCR technology offers opportunities for integrated testing approaches, including simultaneous screening for other sexually transmitted infections (STIs) such as gonorrhoea and syphilis. Overall, the significance of CT screening among MSM using real-time PCR underscores the importance of comprehensive strategies to improve CT detection rates, reduce transmission, and ultimately enhance the health outcomes of MSM populations.

Table 1: Primer and Probe sequence.

OLIGO	PRIMER SEQUENCE
Primer (F)	GAC ACC AAA GCG AAA GAC AAC AC
Primer (R)	ACT CAT GAA CCG GAG CAA CCT
ABI – Probe	[FAM]-CCA CAG CAA AGA GAC TCC CGT AGA CCG-[TAMRA]

F: Forward; R: Reverse; ABI: Applied Biosystems

Table 2: Demographic Characteristic of the study population.

AGE:	NUMBER	PERCENT
9-18	2	1.9
19-28	56	54.36
29-38	35	33.98
39-48	7	6.79
49-58	2	1.94
59-68	1	0.97
MARITAL STATUS		
Married	79	76.6
Unmarried	15	14.56
Separated/Divorced	9	8.73
OCCUPATION:		
Student	22	21.3
Daily Workers	12	11.6
Private	58	56.3
Government	2	1.94
Unemployed	9	8.73
RELIGION:		
Hindu	91	88.3
Christian	11	10.6
Muslim	1	0.97
PERSONAL HEALTH HISTORY:		
Smoking	22	21.35
Alcohol	27	26.21
Tattooing	12	11.65
SEXWORKERS	3	2.9
NON-SEXWORKERS	100	97
SEXUAL ORIENTATION		
Homosexual	74	71.8
Bisexual	29	28.1
TYPE OF SEXUAL ACTIVITY:		
Ororeceptive	78	75.7
Oroinsertive	76	73.7
Anoreceptive	71	68.9
Anoinsertive	68	66
Penovaginal	4	3.88
PROTECTED SEX	8	7.76
UNPROTECTED SEX	95	72.23
MEDICAL HISTORY OF MSM:		
Syphilis	11	10.67
HPV	1	0.97
HSV	4	3.88
HIV	3	2.91

Gonococcal Urethritis	1	0.97
STI PAST HISTORY		
Yes	20	19.41
No	83	80.5

Table 3: *Ct* value of the positive samples.

Positive sample number	*Ct* Value
GNAS7	22.5
GNAS18	29.3
GNAS98	28.4
GNAS102	31.9

Figure 1: Previous history of STI in the study population.

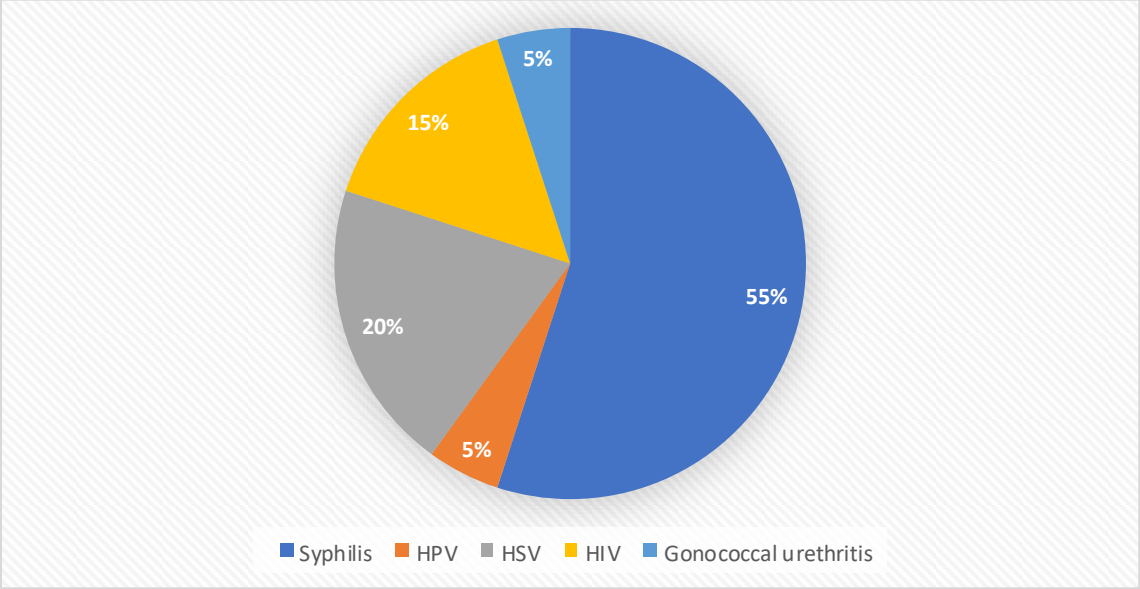
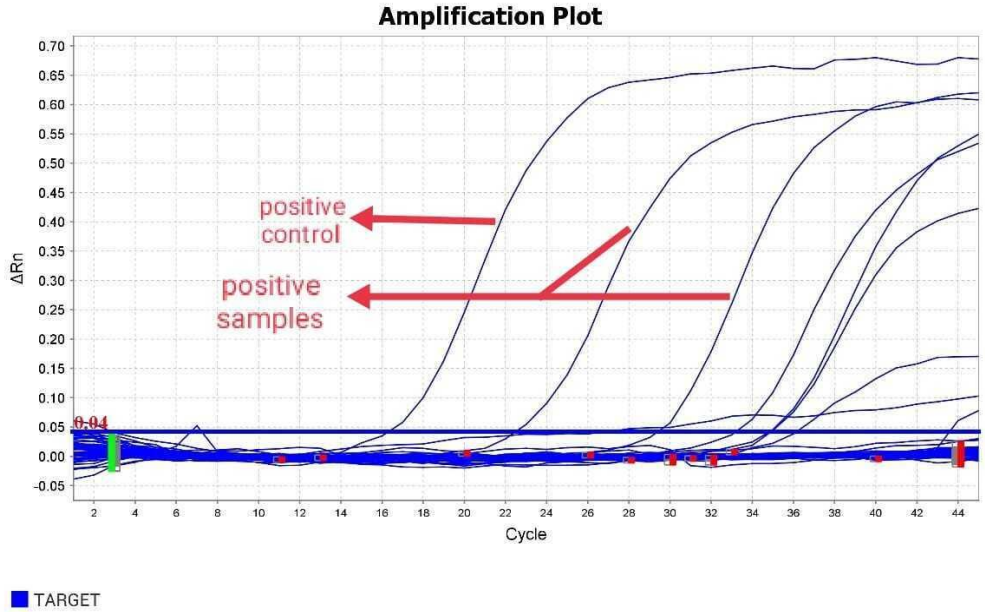
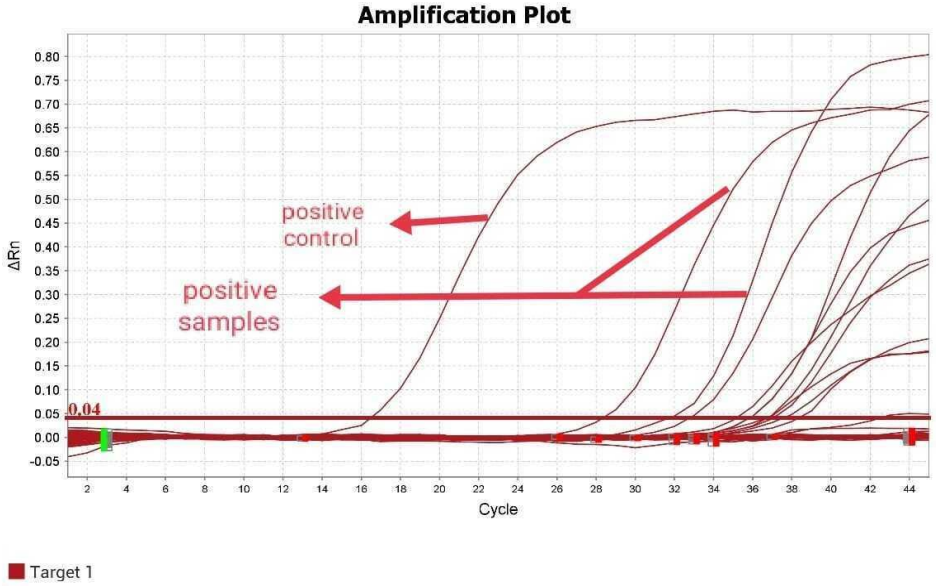


Figure 2: Real Time Polymerase Chain Reaction Results of the study population.



Amplification Plot of sample number 1 to 52.



Amplification plot of samples 53 to 103.

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