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Detection of genital chlamydial and gonococcal infection using urine samples: A community-based study from India

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ABSTRACT

Sexually transmitted infections (STI) have a major impact on the reproductive health of women. Among the different etiological agents of STIs, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the main bacterial pathogens that cause sexually transmitted infections in women. The aim of the study was to estimate the prevalence of genital chlamydial and gonococcal infection among women in the age group of 18–65 years from a community-based setting. A community-based cross-sectional study was performed using the archived urine samples (n = 811) of women in the age group of 18–65 years for *C. trachomatis* and *N. gonorrhoeae* using a multiplex conventional Polymerase Chain Reaction (PCR). Out of 811 samples tested in the present study, 2 (0.24%) were tested positive for *C. trachomatis* and none were positive for *N. gonorrhoeae*. The study demonstrates the very low prevalence of *C. trachomatis* and *N. gonorrhoeae* infection in a rural community. For large population-based screening, urine samples were observed to be more socially acceptable and cost-effective.

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Introduction

Sexually transmitted infections (STIs) have a major impact on the reproductive health of women. Among the ten leading infectious diseases that require notification of the Centre for Disease Control and Prevention (CDC) in the United States (US), five are transmitted sexually. These five infections include genital chlamydial infection, gonorrhea, Human Immunodeficiency Virus (HIV) infection, syphilis, and hepatitis B. The World Health Organization (WHO) reports 101 million chlamydial infections annually [1]. In 2012, among women aged 15–49 years, the estimated global prevalence of chlamydia and gonorrhea was 4.2% and 0.8% respectively. Among men, estimated prevalence of chlamydia was reported to be 2.7% and gonorrhoea 0.6%. Worldwide, 131 million new cases of chlamydia (100–166 million) and 78 million cases of gonorrhoea (53–110 million) were reported in 2012 [2]. The control of sexually transmitted infections is a challenging public health problem in India due to the underreporting of symptoms, associated stigma and irrational use of antibiotics [3]. Karnataka State, in southern India, is having a well-developed healthcare infrastructure and the prevalence of sexually transmitted infections is generally low [4]. Once infected, about 80% women and 50% men are asymptomatic leading to increased reproductive morbidity if left untreated [5]. Moreover, the asymptomatic nature of the disease paves way to the easy transmission of infection among the partners. Undetected or multiple infections in women can lead to serious reproductive sequelae, like ectopic pregnancy and tubal infertility [6].

Chlamydia trachomatis is considered a frequent cause of pelvic inflammatory disease (PID) and infertility worldwide [7]. The early diagnosis and treatment are crucial in avoiding further complications associated with PID. Gonococcal infections are often symptomatic and early gonococcal infections in women and children are subclinical which further spreads to upper genital tract [8]. About 10–20% untreated women with gonorrhoea or chlamydial infection develop pelvic inflammatory disease [9]. The pelvic inflammatory disease leads to tubal scarring altering the tubal anatomy and an estimate of 20% women with PID suffers from infertility, 9% from ectopic pregnancy, and 18% from chronic pelvic pain [10].

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Table 1
Primers used for screening of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Organism	Target gene	Name	Oligonucleotide sequence (5′–3′)	Amplicon size
Chlamydia trachomatis	Cryptic plasmid	CTF1	TCCGGAGCGAGTTACGAAGA	241 bp
		CTR1	AATCAATGCCCGGGATTG	
Neisseria gonorrhoeae	cppB gene	NGF1	GCTACGCATACCCGCGTT	390 bp
		NGRI	CGAAGACCTTCGAGCAGACA	

The fact that *C. trachomatis* and *N. gonorrhoeae* can be a cause of the silent epidemic, emphasises the need for screening, early diagnosis, and treatment of cases and partners [11]. Gonococcal infection is re-emerging as a potent menace to public health due to the high morbidity and multidrug resistance [12,13]. Similar to chlamydial infections, the untreated gonococcal infection can also lead to serious sequelae. Disseminated gonorrhoea results in petechial or pustular skin lesions, asymmetrical arthralgia, tenosynovitis, or septic arthritis [8]. Studies have shown a higher predilection of Chlamydia and Gonococci to the urinary tract of men and women. *N. gonorrhoeae* and *C. trachomatis* are well-established pathogens causing urethritis in both the sexes. Non-Gonococcal Urethritis (NGU) is caused by *C. trachomatis* in 15%–40% of cases [14]. Most of the infections are asymptomatic and individuals with infections often present with long-term sequelae like ectopic pregnancy and infertility. Thus, a syndromic approach will be inadequate to assess the magnitude of the problem.

The development of molecular techniques has dramatically transformed the diagnosis of sexually transmitted infections. But the major hurdle for early detection of these STIs among asymptomatic women is the collection of vaginal and endocervical smears [15]. The answer to this vexed problem lies in the use of noninvasive samples such as urine and self-collected vaginal samples in the screening of asymptomatic women [16]. During the early 1990's techniques of nucleic acid amplification such as Ligase Chain Reaction (LCR) and Polymerase Chain Reaction (PCR) were used to detect *C. trachomatis* in urine samples [10,11].

There is a scarcity of population-based studies from this part of India and we decided to test for Chlamydia and Gonococci in archived urine samples of women in the age group of 18–65 years collected by house-to-house visit. The estimation of the baseline prevalence of chlamydial and gonococcal infection in a community-based setting may be helpful in implementing appropriate evidence-based preventive measures.

Materials and methods

Study design

A cross-sectional study was carried out using urine samples archived at Department of Virus Research (Manipal Centre for Virus Research), Manipal University as part of a community-based Human Papillomavirus study among asymptomatic women in the age group of 18–65 years in a village in Udupi Taluk of Karnataka [17,18]. Soon after collection, the samples were transported in cold chain (4–8 °C) to the laboratory and modified aliquoting was performed on the same day [19]. The samples were immediately stored at −70 °C and well preserved. The minimum sample size of 811 was calculated with the assumption that the expected prevalence of both *C. trachomatis* and *N. gonorrhoeae* as 5% at 95% confidence interval and 1.5% relative precision [20]. The epidemiological information of the participants was collected from data collection sheets after taking written informed consent. We decided to include urine samples from unmarried women also as it was difficult to get a proper sexual history if they are sexually exposed in conservative household settings. All the socio-demographic data and clinical features of the study participants were entered into Epi Info™ 7 and statistical analysis was performed.

Sample aliquoting and processing

About 20–25 ml first void (first stream) urine was collected and transported to the laboratory at 4–8 °C. Urine samples were centrifuged at 3800g for 20 min at 4 °C (Sorvall Legend XTR Thermo Fisher Scientific, Germany). The middle opaque layer was collected, transferred to a 1.5 ml microcentrifuge tube and centrifuged at 16,000g for 15 min at 4 °C. The pellets obtained from this were resuspended in PBS, aliquoted and stored at −20 °C to −80 °C.

DNA extraction and PCR

DNA extraction was performed using QI Amp DNA mini kit (Qiagen, Hilden, Germany) following manufacturer's instructions. We used conventional PCR for detection of *C. trachomatis* and *N. gonorrhoeae* in urine samples as described by Mahony et al. Table 1 shows primers (Sigma–Aldrich Co., LLC, USA) used in the study [21].

The PCR reaction was set up as described by JB Mahony et al. with modifications in the reaction condition [21]. In brief, the reaction mixture of 25 μl containing 5 μl of eluted DNA, 20 μl of a master mix containing 12.5 μl of buffer mix, 2.5 μl of each primer, 1 μl of enzyme mix and 1.5 μl of nuclease free water was used. Extracted DNA from culture isolates for both Chlamydia and Gonococci were used as positive controls. Standardisation of primers was performed using the extracted DNA in different dilutions. Amplification was carried out in Veriti® Thermal Cycler (Life Technologies, USA) with the following cycling parameters; 10 min at 95 °C followed by 40 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C and a final step of 5 min at 72 °C. PCR products were analysed by gel electrophoresis with 1.8% agarose and ethidium bromide staining. The possible PCR inhibition by the urine was tested using the spiking experiment. Urine samples routinely received at Manipal Centre for Virus Research was spiked with extracted chlamydial DNA and tested for chlamydia by the above standardised protocol and all the spiked urine samples were tested positive.

Ethical clearance

The study was reviewed and approved by the Institutional Ethical Committee (IEC), Kasturba Medical College, Manipal University (Reg No. ECR/146/Inst/KA/2013).

Results

A total of 811 archived urine samples were tested for *C. trachomatis* and *N. gonorrhoeae*. The mean age of the study participants enrolled in this community study was 39.5 years (SD = 12.9). The study participants included 599 married (73.86%), 126 unmarried women (15.5%), 76 widowed (9.4%), 9 separated (1.1%) and one divorced lady (0.1%). The high female literacy rate of 86.2% was observed among the study participants. The majority of the study population were homemakers (54.0%). Most of the working women were involved in the cashew industry (17.6%) and 80 women were doing beedi (thin Indian cigarette filled with tobacco flake) rolling

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