**Chlamydia trachomatis** serovar G infection in a bisexual male with urethritis

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**ABSTRACT**

We report a case of *Chlamydia trachomatis* serovar G urogenital tract infection in a 33-year-old human immunodeficiency virus-1 (HIV-1) seropositive Indian bisexual male. This case highlights the emergence of a new serovar in India. The patient was tested positive for *C. trachomatis* by both cryptic plasmid and ompA gene polymerase chain reaction (PCR). On further characterization using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) and ompA gene sequencing, the strain was found to be *C. trachomatis* serovar G. His spouse was also found to be infected with *C. trachomatis* serovar G. Phylogenetic analysis was performed on the clinical isolates obtained from both partners and were found to be identical to the isolates available in GenBank. The sexual network could not be traced further. Detection of a new genotype suggests importation of a new strain into the population probably by sexual contact with a person from a geographical area where the strain is common. Identifying circulating genotypes in the community can assist in developing strategies for improved sexually transmitted disease control.

**Key words:** *Chlamydia trachomatis*, restriction fragment length polymorphism, sequencing

**INTRODUCTION**

Genital infections with *Chlamydia trachomatis* (serovars D-K) are associated with urethritis, pelvic inflammatory disease and infertility.[1] The major outer membrane protein of *C. trachomatis* exhibits extensive DNA sequence variation in its gene (ompA) where the four highly variable domains (variable domain I–IV) of the protein are exposed to the surface. Change in amino acid sequences in variable domain region accounts for the serologic differences among *C. trachomatis* serovars. So far, 19 different serovars of *C. trachomatis* have been identified. Serovars D-K are predominantly isolated from the urogenital tract. As increasing number of isolates have been typed worldwide, transmission between sexual partners have been studied and geographic variation in distribution of serovars has been found. In a worldwide survey on major outer membrane protein's evolutionary dynamics, the most prevalent serovars reported were E followed by F and L2. While the highest ompA variance was found in genotypes L2 and G, genotypes E and F were the least variable.[2] Previous studies from India have shown serovar D to be the most predominant serovar among patients with urogenital infections, followed by E, F and I.[3,4]

We report a case of a bisexual male with genital tract infection with *C. trachomatis* serovar G, a serovar not previously reported from India.

**CASE REPORT**

A 33-year-old man presented to the sexually transmitted disease clinic at All India Institute of Medical Sciences, New Delhi, with a mucopurulent urethral discharge and burning micturition for...
20 days. He was detected to be seropositive for human immunodeficiency virus-1 (HIV-1) one year previously and was on antiretroviral therapy since then. He was bisexual with multiple casual partners and a single marital heterosexual partner. He had engaged in unprotected peno-anal insertive sex with his male partners and peno-vaginal sex with his wife. He stated that he had not been a receptive partner and his perianal orifice did not appear patulous. Since the patient was asymptomatic and denied orogenital contact, he did not permit pharyngeal swab sampling. He refused rectal examination and a rectal swab could also not be taken for microbiological analysis.

The review of laboratory records revealed that the patient had a CD4+ lymphocyte count of 88/μl. He was nonreactive for venereal disease research laboratory (VDRL) test and seronegative for both hepatitis B surface antigen and anti-hepatitis C antibodies. Microscopic examination of Gram-stained smear of the discharge revealed >10 pus cells per oil-immersion field. No Gram-negative intracellular diplococci were seen. Urethral swabs were subjected to culture for *Neisseria gonorrhoeae*, *Ureaplasma* spp. and *Mycoplasma hominis*. Polymerase chain reaction (PCR) assays were undertaken for *C. trachomatis* targeting cryptic plasmid using primers KL1 5’-TCC GGA GCG AGT TAC GAA GA-3’ and KL2 5’-AAT CAA TGC CCG GGA TTG GT-3’ and ompA gene using primers NLO 5’ATG AAA AAA CTG TTG AAA TCG3’ and NRO 5’CTC AAC TGT AAC TGC GTA TTT3’.[5-8] A multiplex polymerase chain reaction (multiplex-PCR) targeting the urease gene of *Ureaplasma* spp. using primers U4 5’ACG ACG TCC ATA AGC AAC T3’ and U5 5’CAA TCT GCT CGT GAA GTA ATT AC 3’ and the 16Sr RNA of *M. hominis* using primers RNAH1 5’CAA TGG CTA ATG CCG GAT ACG C3’and RNAH2 5’GGT ACC GTC AGT CTG CAA T3’ was performed.[9] A polymerase chain reaction (PCR) assay was also performed to detect *M. genitalium* by targeting the 140 kDa adhesion gene using primers MgPa-1 5’AGT TGT GAA ACC TTA ACC CCT TG G3’ and MgPa-3 and 5’CCG TTG AGG GGT TTT CCA TTT TTG C 3’.[10]

Cultures were positive for *Ureaplasma* spp. susceptible to azithromycin, doxycycline, ofloxacin, ciprofloxacin, levofloxacin and josamycin. Polymerase chain reaction (PCR) assay for *Ureaplasma* spp. was also found to be positive and the isolate was further biotyped and serotyped.[11] It was found to belong to biovar 1 (*Ureaplasma parvum*) and serovar 3. The urethral specimen tested positive for *C. trachomatis* by both cryptic plasmid and ompA gene polymerase chain reaction (PCR).

Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) [Figure 1a] and ompA gene sequencing (BigDye Terminator v3.1 cycle sequencing kit, Applied Biosystems, Foster City, CA, USA) were done to characterize the *C. trachomatis* strain and it was found to be serovar G (GenBank KM 504512).[10] The control for serovar G used in the study was kindly provided by Dr. S.M. Bruisten (department of infectious diseases, public health laboratory, Nieuwe Achtergracht 100, Amsterdam).

Contact tracing was attempted but we were only able to test his wife who was seropositive for human immunodeficiency virus 1 (HIV-1). She was heterosexual and monogamous and denied any history of premarital or extramarital sex. Examination revealed a mucopurulent vaginal discharge. Endocervical swabs were collected and she too was found to be infected with *C. trachomatis* serovar G (GenBank KP 015825) and *U. parvum* serotype 3/14.

Phylogenetic analysis was performed by using the maximum-likelihood method implemented in MEGA6 program. Phylogenetic neighbor-joining tree was constructed using the nucleotide sequences based on the *C. trachomatis* ompA nucleotide sequences from both our clinical isolates and nine isolates available from GenBank. As expected, the two clinical isolates
of serovar G were identical. Both the isolates were also identical to the isolates obtained from GenBank. Branch lengths were proportional to the amount of sequence that diverged between taxa in the tree as illustrated by the bar. Relevant bootstrap values (as percentage of 500 replicates) were given [Figure 1b].

Detection of the same *C. trachomatis* genotype in the wife suggested likely acquisition of the infection from her spouse. Both our patients were treated with azithromycin and evaluated 2 weeks later. Post treatment culture and polymerase chain reaction (PCR) assays for *C. trachomatis, and Ureaplasma* were negative. Both the patient and his spouse were counseled for safe sex practices and were advised for follow-up after 2 weeks.

**DISCUSSION**

We were unable to find any previous reports of infection with *C. trachomatis* serovar G from India. Serovar G has been previously identified mainly from European countries and Australia and is common worldwide, especially among men who have sex with men.[12,13] Detection of this genotype suggests importation of a new strain into the population that may have occurred by sexual contact with a person from a geographically distinct area. As our evaluation did not include questions about the ethnicity/origin of sexual partners, this hypothesis could not be proven. Furthermore, the possibility of our patient acquiring *C. trachomatis* serovar G infection from an Indian sexual partner cannot be ruled out on account of the paucity of epidemiological data on the distribution of *C. trachomatis* genotypes in Indian patients. Since serovar G has been reported to occur worldwide, it is possible that the isolate might already be circulating in the Indian population including men who have sex with other men.

Genotyping of *C. trachomatis* is important to understand the population genetic structure and is a useful tool in epidemiological studies, investigation of infection transmission and surveillance of emerging genotypes in populations. It is assumed that persons infected by the same chlamydial genotype are more likely to be epidemiologically linked than those infected with a different genotype. Of note, *ompA* is a fast evolving gene and is under strong selective pressure. Globally, the adapted evolution of the *C. trachomatis* dominant antigen is likely driven by its complex pathogenesis related function and reflects distinct evolutionary antigenic scenarios that may benefit the pathogen.[2] Phylogenetic studies have shown that *ompA* shows extensive recombination and in many instances is a chimera that can be exchanged in parts or whole, both within and between biovars.[14]

In addition to polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP), we used *ompA* gene sequencing to confirm the characterization of the strains of *C. trachomatis* detected from both the patients and to link molecular information for contact tracing. Phylogenetic analysis was performed on both our clinical isolates. As expected, the two clinical isolates of serovar G were identical. Both the isolates were also identical to the isolates obtained from GenBank. This provides strong evidence for sexual transmission of the strain but we were unable to examine other sexual contacts of our patient. The multiplicity of his sexual partners makes it likely that this serovar would be transmitted to others in the community.

Studies on chlamydial infection have identified a variety of risk factors including the number of partners, age under 25 years, concurrent gonococcal infection, a history of sexually transmitted diseases, human immunodeficiency virus (HIV) seropositivity and seroconversion and the lack of condom use.[15,16] In our patient, multiple cofactors were present such as multiple casual sexual partners, presence of other sexually transmitted disease’s viz., *U. parvum*, human immunodeficiency virus-1(HIV-1) and lack of condom use.

Genital *C. trachomatis* infections (caused by serovars D through K) have been recognized as the most prevalent bacterial sexually transmitted infections throughout the world. Chlamydial infections lead to non-gonococcal urethritis and cervicitis which, if undiagnosed and not treated in a timely manner, may result in serious secondary complications and sequelae including pelvic inflammatory disease, ectopic pregnancy, tubal infertility and increased risk of human immunodeficiency virus (HIV) transmission and acquisition. Considering the high rate of asymptomatic chlamydial infection, particularly in women, a substantial “silent” or undetected epidemic of *C. trachomatis* infections could put this population at significant risk for human immunodeficiency virus (HIV) infection. Epidemiological studies
have shown that sexually transmitted pathogens including agents that do not cause genital ulcers, such as *Chlamydia trachomatis* and genital mycoplasmas may serve as biological cofactors and act in synergy with human immunodeficiency virus (HIV) and exacerbate retroviral disease.\(^{17}\)

The identification of this new serovar indicates the need for larger epidemiological and clinical studies in India as characterization of *Chlamydia trachomatis* strains can provide important epidemiological knowledge and contribute to improved control measures.

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**Conflicts of interest**

There are no conflicts of interest.

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