Short Communication

Detection of Chlamydia trachomatis by Immunological and Genetic Methods in Female Sex Workers and the Local Female Population of Reproductive Age in Mymensingh, Bangladesh

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SUMMARY: To investigate the accurate prevalence of genital Chlamydia trachomatis infection in Mymensingh, a local area in central-northern Bangladesh, 40 female sex workers (FSW) and 110 sexually active women (SAW, non-FSW) of reproductive age from a local community with clinical symptoms were examined by an immunochromatography test (ICT) and plasmid-based polymerase chain reaction (PCR) during a 1-year period from July 2011 to June 2012 using the endocervical swab as a specimen. By ICT and/or PCR, the C. trachomatis detection rate was 58% and 27% in FSW and SAW, respectively, showing a significant difference (P < 0.01). Two C. trachomatis strains from FSW were determined to be serovar D by ompA-based PCR and sequencing analysis. The highest prevalence was found among women aged 15 to 35 years. A lower socioeconomic status was considered to be an important risk factor for C. trachomatis infection in FSW but not in SAW. This is the first study to determine the prevalence of C. trachomatis infections in FSW and SAW in the same local area in Bangladesh.

Genital infections with Chlamydia trachomatis are now recognized as a highly prevalent type of sexually transmitted diseases (STDs). In terms of frequency, they surpass the classic STDs such as syphilis and gonorrhea and thus constitute a serious public health problem. According to the World Health Organization (WHO) global estimates in 1999, the four major STDs among people aged 15–49 years were syphilis, gonorrhea, chlamydial infection, and trichomoniasis, and the total number of new cases of these curable STDs was approximately 340 million (1). Among these, chlamydial incidence was approximately 92 million (27%). The largest number of new cases (42.89 million) occurred in the region of South and Southeast Asia, accounting for 47% of the total infected cases. The prevalence of C. trachomatis infection diagnosed by polymerase chain reaction (PCR) using genital specimens among women in India was reportedly 23%–32% (2,3). In Bangladesh, there have been some studies on the prevalence of C. trachomatis infections, which examined only female sex workers (FSW) in Dhaka or other cities (4–7). In these studies, the prevalence of C. trachomatis infection was reported to be 16%–44%. However, there is little information on C. trachomatis infections in the general population in the local community, or in local areas other than the capital city Dhaka. Previously, in Mymensingh, an area located in central-northern Bangladesh, the detection of C. trachomatis antigens was attempted for women who attended the Mymensingh Medical College Hospital, revealing positive rates of 45% by immunological tests (8) and 30% by PCR (9). However, in these studies, different detection methods were used for an insufficient number of subjects and a difference in prevalence between FSW (high-risk group) and non-FSW (sexually active women [SAW]) (low risk group) was not evident. Therefore, the present study was designed to accurately determine the prevalence of C. trachomatis infections among the female population from these groups in Mymensingh.

Most C. trachomatis strains have an extrachromosomal plasmid (cryptic plasmid) that is approximately 7.5 kb in size. This C. trachomatis plasmid is a favored target for DNA-based diagnosis of C. trachomatis infection because there are multiple copies of this plasmid in a single bacterial cell (10). PCR analysis has been introduced to detect C. trachomatis because of its high sensitivity (11,12). The major outer membrane protein (MOMP) is the principal immunodominant surface antigen of C. trachomatis. The MOMP has antigenic determinants located across four symmetrically spaced variable domains that are flanked and interspaced by five constant domains. The ompA gene encodes the variable domains and their nucleotide sequences exhibit distinct variations in 19 different serovars (10). Thus, they have been used both for detecting and genotyping C. trachomatis isolates (12,13). Genotypic characterization of C. trachomatis isolates provides not only valuable insights into the C. trachomatis serovars that are...
circulating within a given community but also sheds light on their global epidemiology, which may assist in developing strategies for improved STD control (14). An immunochromatography test (ICT) that includes monoclonal antibodies to detect lipopolysaccharide antigens extracted from C. trachomatis isolates can be used as a screening test because of its high sensitivity (94%) and specificity (99%) (15). In the present study, for confirmatory detection of C. trachomatis, PCR targeting for the cryptic plasmid and ICT were used to examine endocervical swab specimens, and serovars were determined by sequencing analysis of the ompA gene.

Specimens were collected from 150 subjects. They included 110 local women of reproductive age (<45 years old) who had visited the Department of Gynecology and Obstetrics, Mymensingh Medical College Hospital, during the period from July 2011 to June 2012 with complaints of chlamydial infection-like clinical symptoms. For this study, they were referred to as SAW. The 40 other subjects were brothel-based FSW who were aware of STDs and agreed to participate in this study. Their specimens were obtained during the same period as that for SAW with the cooperation of a non-governmental organization that took care of them. For each subject, data were recorded on a structured sheet during interview. Our study protocol was reviewed and approved by the Institutional Review Board of Mymensingh Medical College. Verbal informed consent was obtained from all the subjects following the guidelines of the ethical review committee of Mymensingh Medical College.

A sterile Dacron swab stick was used to collect endocervical secretions for ICT and a cytobrush was used for PCR. The Dacron swab stick was placed into a sterile tube containing Tris–EDTA buffer (10 mM Tris, 1 mM EDTA). For ICT, Clearview® Chlamydia MF kits (Inverness Medical Innovations, Inc., Waltham, Mass., USA) were used according to the manufacturer’s instructions. To extract a DNA sample for PCR analysis, a tube containing 500 μl of Tris–EDTA buffer along with cytobrush was centrifuged at 12,000 rpm for 3 min, following which 300 μl of the supernatant was discarded. The remaining buffer was used to resuspend the precipitate. This mixture was incubated at 100°C for 15 min and then centrifuged at 10,000 rpm for 1 min. The supernatant was used as a DNA template, as described previously (16). PCR was used to amplify a 517-bp fragment from the common endogenous plasmid as described previously, using a pair of oligonucleotide primers, T1 and T2 (11). Nested ompA-based PCR was used to obtain a 965-bp product with the primer pairs 29CHOMP and CHOMP336, as described previously (13). The nucleotide sequences of ompA-based PCR products from clinical samples of patients with chlamydial infection were determined using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA) with an automated sequencer (ABI PRISM 3100; Applied Biosystems).

Table 1 shows the numbers and rates of C. trachomatis infections detected in this study. The amplified PCR products from some specimens are shown in Fig. 1. The overall C. trachomatis detection rate with PCR (33%) was significantly higher than that with ICT (26%) (P = 0.013, by exact binomial test; P < 0.05, McNemar’s test with continuity correction). Of the 150 total subjects, C. trachomatis was tested positive in 53 specimens (35%) by either ICT or PCR. The detection rates in FSW were 40%, 50%, and 58%, by ICT alone, PCR alone, and either ICT or PCR, respectively. These were significantly higher than those in SAW (21%, 28%, and 28%, respectively). Three specimens were positive by ICT only, and 14 were positive by PCR only. From sequencing analysis, amplified PCR products (965 bp) of the ompA gene in two specimens from FSW proved to have sequences identical to that of the C. trachomatis reference strain, 181C/91 (GenBank accession no. FJ943517), which belongs to serovar D. Most

Table 1. Detection of C. trachomatis by ICT and PCR in FSW and SAW

<table>
<thead>
<tr>
<th>Category of patients</th>
<th>No. of patients</th>
<th>No. of positive specimens (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ICT</td>
<td>PCR</td>
</tr>
<tr>
<td>FSW</td>
<td>40</td>
<td>16 (40)*</td>
</tr>
<tr>
<td>SAW</td>
<td>110</td>
<td>23 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>39 (26)</td>
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* , **: Significantly higher rate than SAW (*, P < 0.05; **, P < 0.01).
of the *C. trachomatis*-positive subjects (83% FSW and 90% SAW) were younger than 35 years of age (17–35 years), and nearly half of the FSW and SAW with *C. trachomatis* infections were younger than 25 years of age. *C. trachomatis*-positive cases (ICT or PCR) in FSW and SAW mostly had complaints of excessive vaginal discharge (81%) and lower abdominal pain (70%), followed by dysuria (53%), and inter-menstrual bleeding (6%). Among FSW and SAW, 69% and 23% of the cases with *C. trachomatis* infections, respectively, were from a lower socioeconomic group, which was defined as a family with a monthly income of ≤3,000 TK (approximately 43 US$), and this difference was statistically significant (*P* < 0.05).

In our analysis, cryptic plasmid-based PCR detection was negative for the three ICT-positive specimens. One possible reason for the negative PCR results was the presence of inhibitors in these specimens, which is well known to occur for nucleic acid amplification tests (17). Another possibility was the presence of genetic *C. trachomatis* variants that lacked the endogenous plasmid (18,19), although the occurrence of such variants in this study area was not evident. However, the PCR analyses of these negative specimens may not have included the new Swedish variant of *C. trachomatis* that has an internal 377-bp deletion in coding sequence 1 (CDS1) of its plasmid (20,21), because the PCR target gene in the present study (primers T1 and T2) was CDS3.

This is the first study to report the prevalence of *C. trachomatis* infections in FSW and SAW living in the same local area in Bangladesh. Our *C. trachomatis* detection rate in FSW (58%) was higher than that reported previously for FSW in Dhaka and Rajshahi City, Bangladesh (16–44%) (4–7). However, the higher rate observed in the present study may have resulted from the use of two highly sensitive detection methods, or from the lower number of FSW subjects (*n* = 40) in the present study settings in a rural area. Notably, the *C. trachomatis*-positive rate in SAW (35%) was comparable with previously reported rates in FSW in Dhaka (4, 6,7), suggesting an increase in *C. trachomatis*-infected individuals in the general populations outside Dhaka. In contrast to the higher rate of subjects in the lower socioeconomic status in FSW (69%), more than 70% of SAW with *C. trachomatis* infections were not related to this socioeconomic status. This suggests that *C. trachomatis* infections are common not just among poor people in Bangladesh. Thus, it may also be important to provide knowledge about *C. trachomatis* infection and its prevention to populations living in rural areas.

As shown in the present study and as reported previously (17), PCR analysis revealed a higher *C. trachomatis* detection rate than ICT. However, when *C. trachomatis* screening is introduced in Bangladesh, PCR analysis will be more expensive to perform than ICT, both in terms of the reagents required as well as the establishment of sophisticated laboratories and the necessary equipments. In contrast, ICT does not require any special facilities, and can be performed by any laboratory technician without any specific experimental skills, and in a considerably shorter time. The specificity of ICT is known to be very high (22), and ICT can be used to detect *C. trachomatis* irrespective of genetic plasmid variations. Despite its slightly lower sensitivity than PCR, ICT will be recommended as one of the practical options for *C. trachomatis* screening, or as a complementary diagnostic method in Bangladesh. Genetic methods such as PCR should be occasionally employed for research purposes to investigate the distributions of serovars, the presence of *C. trachomatis* variants and others.

In conclusion, the results of our study indicated a high prevalence of *C. trachomatis* infection in Mymensingh, one of the local areas in Bangladesh, in SAW as well as FSW by two confirmatory tests. These tests provided evidence for the presence of *C. trachomatis* serovar D in this area. Continuous epidemiologic surveillance, and effective control measures for *C. trachomatis* infections for FSW, and SAW in the general population are considered to be necessary in Bangladesh.

**Conflict of interest** None to declare.

**REFERENCES**


