Original Article

Prevalence and Genotype Distribution of *Chlamydia trachomatis* in Urine among Men Attending Sexually Transmitted Disease Clinics in Guangdong Province, China, in 2016

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SUMMARY: Studies have rarely assessed the genotype distribution of *Chlamydia trachomatis* (CT) in urine among men attending sexually transmitted disease clinics (MSCs) in China. This study was aimed at investigating the prevalence and molecular epidemiology of CT infection by examining urine samples among MSCs from different geographic areas of Guangdong Province, China. A cross-sectional study was conducted among MSCs from 10 human immunodeficiency virus sentinel surveillance sites in Guangdong Province. CT DNA was extracted from male urine samples and analyzed using a Roche cobas 4800 CT/NG. The *ompA* genes were amplified by nested PCR and sequenced. The leukocyte esterase test was performed by routine urine analysis at local clinics. Of the 1,903 samples, 163 (8.6%, 95% confidence interval [CI] 3.8–16.3%) tested positive for CT. The highest prevalence (10.5%) of CT infection was observed among participants aged between 21 and 30 years. A total of 130 CT-positive samples (79.8%, 130/163) were successfully genotyped by nested PCR, resulting in 8 genotypes. The most prevalent genotypes were D, E, F, and J, with proportions of 20.8%, 20.0%, 17.7%, and 16.9%, respectively. There were no significant correlations between the geographical areas, leukocyte esterase test results and genotype distribution. Promotion of detection and molecular epidemiology research is needed for effective and comprehensive prevention and control programs.

INTRODUCTION

Globally, *Chlamydia trachomatis* (CT) infection is the most common sexually transmitted disease (STD). Although it is curable, CT infection is still a serious public health problem in both developing and developed countries (1,2). If untreated, it can lead to a host of serious complications and reproductive health problems, such as infertility, ectopic pregnancy, and pelvic inflammatory disease (3). World Health Organization (WHO) estimates that 131 million new cases of CT occurred among adults and adolescents aged between 15 and 49 years worldwide in 2012, with a global incidence rate of 38 per 1,000 women and 33 per 1,000 men (4). In 2015, a total of 1,526,658 CT infections were reported in the United States (5). The reported incidence rate of

genital CT infection increased from 32.48 per 100,000 people in 2008 to 37.18 per 100,000 people in 2015 at 105 national STD surveillance sites in China, with the average annual rate being 1.95%. Among all age groups, the sexually active population aged between 20 and 29 years showed the highest reported incidence of genital CT infection (6). In Guangdong Province, the incidence of reported cases of CT infection increased from 0.5 per 100,000 people in 2006 to 51.3 per 100,000 in 2014 (7).

Genotyping is an effective technique for epidemiological studies and clinical work on CT infections. The major genotyping methods include restriction fragment length polymorphism (RFLP), fluorescent PCR, PCR-sequencing, and PCR hybridization (8). Many studies have shown feasibility of genotyping by PCR-based sequencing of the amplified *ompA* gene, which encodes major outer-membrane protein (MOMP) (9,10). MOMP is the major immunogenic antigen of CT with 4 variable domains. Variable domains are encoded by the *ompA* gene, and their nucleotide sequence shows distinct variations in different serovars. Thus, the *ompA* gene is a suitable target for genotyping (8–10). The genotypic characters of CT not only can provide valuable CT genotype information within a given community, but also can improve the understanding of the disease epidemiology. Both of these should be useful for the development of STD prevention and intervention strategies.

At present, urethral, endocervical, vaginal, and rec-

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tal samples are commonly used as biological material for CT detection and genotyping (11,12). Collection of urine, however, is much more acceptable to patients and is considered the recommended sample type for men. Urine collection as a non-invasive sampling method has been used for CT screening in developed countries (13,14). Nonetheless, urine is seldom used for CT detection in China (15). In this study, we collected urine from men attending STD clinics (MSCs) and performed CT detection.

The nucleic acid amplification test (NAAT) performed in this study is the most sensitive assay for CT detection (16,17). The concentration of *Chlamydia* decreases during urination but the NAAT can detect low concentrations of CT. Nonetheless, genotyping requires greater amounts of a PCR product for DNA sequencing. Therefore, we used nested PCR to increase DNA concentration for genotyping.

To the best of our knowledge, this is the first study with a relatively large sample size of urine specimens for CT genotyping in Guangdong Province (12). To fill this knowledge gap, we aimed to assess the prevalence of CT among MSCs and to evaluate the genotype distribution of CT.

MATERIALS AND METHODS

Study sites and population: A cross-sectional study was conducted among MSCs from 10 human immunodeficiency virus (HIV) sentinel surveillance sites in Guangdong Province, China. These 10 sites are located in 10 municipal cities in Guangdong Province, namely, Maoming, Jiangmen, Foshan, Duangguang, Shaoguan, Zhanjiang, Shantou, Qingyuan, Zhuhai, and Chaozhou. The 10 sites were subdivided into 3 geographic areas, which included the Pearl River Delta, the western and northern regions of the Province, and the eastern region of the Province.

Written informed consent for participation was obtained from all the participants. The protocol was approved by the ethics committee of the Guangdong Provincial Center for Skin Diseases and Sexually Transmitted Infection (STI) Control and Prevention. MSCs were also provided optional services in counseling and testing for both HIV and syphilis.

Urine samples were collected from 1,926 MSCs between April and August 2016 in Guangdong Province, China. The median age of the participants was 38 years (range, 12–87 years). A total of 47 participants (2.5%) were men who have sex with men. Additionally, 95 (5.0%) tested positive for syphilis, and 15 (0.8%) were positive for HIV. A total of 755 participants were from the Pearl River Delta, 789 were from Guangdong's western and northern regions, and 359 were from Guangdong's eastern region. There were no significant differences in age distribution among the geographic areas ($\chi^2 = 0.04$, $P = 1.00$).

Urine collection: From each eligible participant, 20–30 mL of urine was collected and divided into 2 portions. The first portion was used for the leukocyte esterase test (LET) at local clinics, and the other portion was subjected CT detection at Guangdong Provincial Dermatology Hospital. The urine samples were transported at the appropriate temperatures to the clinical laboratory

for processing and testing. Samples for PCR were stored at 4°C until processing.

DNA extraction and detection of CT: DNA was extracted from the male urine samples and tested for CT and *Neisseria gonorrhoeae* (NG) sequences using a Roche cobas 4800 CT/NG (Roche Diagnostics, Mannheim, Germany). The cobas 4800 system performs a diagnostic assay on an automated workstation to isolate nucleic acids from clinical samples and is a real-time instrument for the detection of CT (6). Experiments were conducted under the guidance of the manufacturer's instructions. The isolated DNA was stored at –20°C until its use for genotyping.

Amplification of the *ompA* gene fragment and sequencing: The *ompA* genes (VS1–VS2) were amplified by PCR according to a previously described method in our laboratory (T100; Bio-Rad, Hercules, CA, USA) (9,18). For the *omp1* VS1–VS2 PCR, the *ompA* gene sequences of 11 different *C. trachomatis* serovars were retrieved from GenBank. DNA fragment VS1–VS2, which is known to encode type-specific epitopes, was selected as the detection target, and nested PCR primers were designed for *ompA* constant regions flanking variable segments VS1–VS2 using DNASTAR software ver. 5.0 (Madison, WI, USA). The outer primers of the primary PCR amplification were CT1 (5'-TGAACCAAGCCTTATGATCGAC-3') and CT2 (5'-CGGAAT TGTGCATTTACGTGAG-3'). The inner PCR primers for the VS1–VS2 were CT3 (5'-ACTTTGTTTTCGACCGTGTGTTTG-3') and CT4 (5'-GATTGAGCGTATTGG AAAGAAGC-3'). The fragments are sized 516 bp and 453 bp. The amplified DNA was sent to Life Technologies (Shanghai, China) for DNA sequencing. The inner forward and reverse primers were used for sequencing and genotyping. The obtained *ompA* sequence was compared with a sequence in the National Center for Biotechnology Information (NCBI) database by means of BLAST searches.

Urine LET: This test is a nonspecific rapid assay for detection of the presence of an esterase enzyme produced by polymorphonuclear leukocyte. LET was performed by the dry chemical method at local clinics (Dirui, Changchun, China) before testing by PCR. Either positive or negative result was obtained for every sample.

Statistical analysis: SPSS software ver. 15.0 (Chicago, IL, USA) was used for statistical analyses. The Chi-square test or the Fisher's exact test (when sample sizes were small) were carried out to compare the proportions between different groups. Data with $P < 0.05$ were considered significant.

RESULTS

Prevalence of *Chlamydia* infection: Out of the 1,926 urine samples, 1,903 (98.8%) were successfully used in valid tests for the detection of CT and NG. A total of 163 (8.6%, 95% confidence interval [CI] 3.8–16.3%) participants were found to be positive for CT (Table 1), and 64 (3.4%, 95% CI 2.6–4.3%) participants tested positive for NG. Among them, 14 were cases of CT and NG co-infection. The highest prevalence of CT infection was observed among participants aged between 21 and 30 years (10.5%). The prevalence of CT infection in

Table 1. Prevalence of *C. trachomatis* in urine from men

Factor	Sample size	No. infected	Infected (%) (95% CI)
Age (yr)			
≤ 20	93	8	8.6 (3.8–16.3)
21–30	488	51	10.5 (7.9–13.5)
31–40	463	37	8.0 (5.7–10.9)
41–50	459	44	9.6 (7.1–12.7)
≥ 51	400	23	5.8 (3.7–8.5)
Total	1,903	163	8.6 (7.3–9.9)
Geographic area			
Pearl River Delta	755	64	8.5 (6.6–10.7)
Eastern region	359	51	14.2 (10.8–18.3)
Western and northern region	789	48	6.1 (4.5–8.0)

CI, confidence interval.

Table 2. Differences between age and genotypes

Age (yr)	B	D	E	F	G	H	J	K
≤ 30	1 (2.1)	12 (25.5)	12 (25.5)	10 (21.3)	5 (10.6)	0 (0)	7 (14.9)	0 (0)
≥ 31	1 (1.2)	15 (18.1)	14 (16.9)	13 (15.7)	12 (14.5)	4 (4.8)	15 (18.1)	9 (10.8)
Total	2 (1.5)	27 (20.8)	26 (20.0)	23 (17.7)	17 (13.1)	4 (3.1)	22 (16.9)	9 (6.9)
χ^2	0.17	1.02	1.41	0.65	0.39	2.34	0.22	5.48
<i>P</i>	0.68	0.31	0.24	0.42	0.54	0.13	0.64	0.02

Table 3. Differences between geographic area with genotypes

Geographic area	B	D	E	F	G	H	J	K
Pearl River Delta	1 (2.0)	6 (12.0)	9 (18.0)	10 (20.0)	10 (20.0)	2 (4.0)	11 (22.0)	1 (2.0)
Eastern region	1 (2.4)	10 (24.4)	7 (17.1)	7 (17.1)	5 (12.1)	1 (2.4)	7 (17.1)	3 (7.3)
Western and northern regions	0 (0.0)	11 (28.2)	10 (25.6)	6 (15.4)	2 (5.1)	1 (2.6)	4 (10.3)	5 (12.8)
Total	2 (1.5)	27 (20.8)	26 (20.0)	23 (17.7)	17 (13.1)	4 (3.1)	22 (16.9)	9 (6.9)
χ^2	1.10	4.00	1.10	0.34	4.30	0.23	2.15	4.00
<i>P</i>	1.00	0.15	0.56	0.84	0.11	1.00	0.36	0.13

Table 4. Differences between leukocyte esterase test and genotypes

LET	B	D	E	F	G	H	J	K	Total
Positive	1 (50.0)	12 (44.4)	13 (50.0)	10 (43.5)	6 (35.3)	1 (25.0)	12 (54.5)	3 (33.3)	58 (44.6)
Negative	1 (50.0)	15 (55.6)	13 (50.0)	13 (56.5)	11 (64.7)	3 (75.0)	10 (45.5)	6 (66.7)	72 (55.4)
Total	2	27	26	23	17	4	22	9	130
χ^2	0.7	0.27	0.38	0.02	0.69	0.09	1.06	0.13	2.90
<i>P</i>	1	0.60	0.54	0.9	0.41	0.77	0.30	0.72	0.89

LET, leukocyte esterase test.

the 21–30 age group was slightly higher than that in the group that was over 50 years old (5.8%, $P = 0.04$). According to the geographic area distribution, the highest prevalence (14.2%) of CT infection was in the eastern region, and different regions had different rates of CT prevalence ($\chi^2 = 20.80$, $P < 0.01$).

Age vs. genotypes: Of the 163 urine samples that tested positive for CT in the Roche PCR assay, the *ompA* gene was amplified by the nested PCR in 132 (81.0%). There was not enough amplified DNA for sequencing for the other 31 samples. Overall, 130 samples were successfully genotyped and showed 8 genotypes. The most prevalent genotypes were D, E, F, and J, with prevalence of 20.8%, 20.0%, 17.7%, and 16.9%, respectively (Table 2). Infection with genotype K was more common among people who were more than 30 years old ($\chi^2 = 5.48$, $P =$

0.02).

Geographic areas vs. genotypes: There were no significant differences in genotypes among geographic areas ($\chi^2 = 14.68$, $P = 0.40$) (Table 3).

Urine LET results vs. genotypes: This LET was performed on all the samples. Among 130 successfully genotyped samples, there was no significant correlation between the genotype and LET results ($\chi^2 = 2.90$, $P = 0.89$) (Table 4).

DISCUSSION

In this study, a high prevalence (8.6%) of CT infection in urine samples among MSCs in Guangdong Province, China, was reported. The highest prevalence was among participants aged between 21 and 30 years. The most

prevalent genotypes were D, E, F, and J. This study extended the existing literature data by assessing CT prevalence among MCSs in urine samples: via genotyping of the samples among Chinese MSCs.

The overall prevalence of CT in this study is higher as compared to that described in other studies in China. The prevalence of CT in urine samples was 5.9% in Jiangsu Province (15). Our prevalence is also higher than the prevalence rates in a study conducted in 3 cities of Guangdong Province: in Shenzhen, Zhuhai, and Qingyuan (5.5%) (18). The CT prevalence rates in India, Russia, and Zimbabwe were 0.9%, 4.9%, and 3.8%, respectively (19). In our previous study, the CT prevalence among MSCs was 6.07% in 9 cities in Guangdong Province in 2015 (20). It is an urgent task to control CT infection in Guangdong Province, China. Moreover, the highest prevalence was detected among people aged between 20 and 30 years in our study. This finding is similar to the results of a study conducted in Guangdong in 2014, which indicated that men aged between 25 and 34 years have the highest incidence of CT (18). The result of this study is also similar to the findings of the U.S. CDC, which reported that in 2015, the highest incidence of CT in the U.S. was among people aged between 20 and 29 years (5). Similar results were also obtained in Europe in 2013 (21).

The prevalence of CT infection appeared to be regionally distributed. The western and northern regions have comparatively low prevalence, while the eastern region has a high prevalence rate. The eastern area of Guangdong Province, the first special economic zone in China, has special cultural traditions. The high prevalence of CT infection may be related to local culture and economic conditions. Comprehensive prevention and control programs—including not only screening and medical care but also behavioral interventions—are essential to meeting the needs of this region's population.

In urine samples, the most prevalent genotypes detected in this study were D, E, F, and J. This genotype distribution is similar to that in our previous study, which also indicated that E, F, J, and D were the most common genotypes among male and female patients in Guangdong Province from 2005 to 2014 (9,22). Another study conducted in Shenzhen revealed similar findings (23). It suggested that the prevalence of genotypes D, E, F, and J is stable in Guangdong Province.

The most prevalent genotypes in our study are similar to those observed in the heterosexual populations of Asian and Western countries. Genotype E was the most frequently identified subtype from the mid-1990s–2000 in Japan (24). Moreover, genotype E was the most prevalent (70.8%) single infection in Tunisia (25). In the remote regions of Australia, the most common genotypes were D, E, F, and K (26). The most predominant genotypes were E, followed by D and F from 2005 to 2007 in South American samples (27).

One case of genotype B, recognized as an ocular serovar and a cause of ocular infection, was detected in our study. It is likely that genotype B can be sexually transmitted in a sustainable manner. Two cases of genotype B were reported by our research team in 2010 (28). There were some pieces of evidence for transmission of trachoma genotypes in sexual networks. Takahashi et al. detected 11 cases of genotype B among Japanese

patients (24). Similarly, 2 cases of genotype B were identified in 1987 and 1996 in Finland, and potential evidence of transmission of trachoma genotypes in sexual networks was identified too (29).

The relation between certain *C. trachomatis* genotypes and virulence remains weak. Although there have been several reports on the possible relation between certain genotypes and clinical features, it is not likely that typing of *ompA* or of *ompA*-encoded protein MOMP in the urogenital strains will reveal clues to their pathogenicity (30–32). Our data indicate that there was no significant correlation between genotype distribution and LET results. Furthermore, we found 9 cases of genotype K among people aged between 30 and 60 years, but there was no genotype K among MSCs younger than 30 years. Whether genotype K is associated with age has not been reported.

There are quite a few limitations of our study. First, the distribution of genotypes may be affected by the small sample size of our research. Second, genotyping of the *ompA* gene is the traditional method for distinguishing CT strains. Recently, multilocus variable-number tandem-repeat analysis (MLVA) and multilocus sequence typing (MLST) were developed for genotyping (33).

Even with these limitations, it can be concluded that the prevalence of CT has been high among MSCs in Guangdong Province, and that D, E, F, and J, are the most prevalent genotypes. Focused interventions are urgently required for controlling the epidemic of CT, specifically the cases in Guangdong's eastern region.

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Conflict of interest None to declare.

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