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

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Key words: *Chlamydia trachomatis*; urine, genotype; men attending STD clinics
SUMMARY  Studies rarely assessed the genotype distribution of Chlamydia trachomatis (CT) in urine among men attending STD clinics (MSCs) in China. This study aimed to investigate 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 ten HIV sentinel surveillance sites in Guangdong province. CT DNA in men urine samples were extracted and detected by using the Roche cobas® 4800 CT/NG. The ompA genes were amplified by nested polymerase chain reaction (PCR) and sequenced. The leukocyte esterase test was performed by routine urine analysis at local clinics. Of the 1903 samples, 163(8.6%, 95%CI 3.8- 16.3%) were found to be positive for CT. The highest prevalence (10.5%) of CT infection was observed among participants aged 21-30. One hundred and thirty CT positive specimens (79.8%, 130/163) were successfully genotyped by nested PCR, resulting in eight 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 was no significant difference in geographic area, leukocyte esterase test 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). Though 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). The World Health Organization (WHO) estimates that 131 million new cases of CT occurred among adults and adolescents aged 15-49 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 in 2008 to 37.18 per 100,000 in 2015 in 105 national sexually transmitted disease surveillance sites in China, with the average annual rate of being 1.95%. Among all age groups, the sexually active population aged 20-29 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 population of 100,000 in 2006 to 51.3 per population of 100,000 in 2014 (7).

Genotyping is an effective technique in epidemiological studies and clinical work for CT infections. The major genotyping methods include restriction fragment length polymorphism (RFLP), fluorescent polymerase chain reaction (PCR), PCR-sequencing, and PCR hybridization (8). Many studies have shown the feasibility of genotyping by using PCR-based sequencing of the amplified \( \text{ompA} \) gene, which encodes the major outer membrane protein (MOMP) (9, 10). The MOMP is the major immunogenic antigen of CT with four variable domains. Variable domains are coded by \( \text{ompA} \) gene, and their nucleotide sequence exhibits distinct variations in different serovars. Thus, the \( \text{ompA} \) gene is a suitable target for genotyping (8, 9, 10). The genotypic characters of CT can not only provide valuable CT genotypes information within a given community, but also can improve the understanding of diseases.
epidemiology. Both of these would be useful for developing STD prevention and intervention strategies.

At present, urethral, endocervical, vaginal, and rectal specimens are commonly used samples for CT detection and genotyping (11, 12). Collection of urine, however, is much more acceptable, and is considered to be the recommended sample type in men. Urine as a non-invasive specimen has been used in CT screening in developed countries (13, 14). However, 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), used in this study, was the most sensitive assay for CT detection (16, 17). The concentration of Chlamydia decreased during urination but the NAAT can detect low concentration of CT. However, genotyping needed more PCR products for DNA sequencing. Therefore, we used nested-PCR to improve DNA concentration for genotyping.

To the best of our knowledge, it is the first time to use 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 evaluate the genotype distribution of CT.

Materials and Methods

Study sites and population

A cross-sectional study was performed among MSCs from ten HIV sentinel surveillance sites in Guangdong province, China. The ten HIV sentinel surveillance sites were located in ten municipal cities in
Guangdong Province, namely Maoming, Jiangmen, Foshan, Duangguang, Shaoguan, Zhanjiang, Shantou, Qingyuan, Zhuhai, Chaozou. The ten sites were divided into three 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 participants. The protocol has been approved by the ethics committee of the Guangdong Provincial Center for Skin Diseases and STIs Control and Prevention. MSCs were also provided voluntary services in counseling and testing for both HIV and syphilis.

Urine samples were collected from 1,926 MSCs between April and August of 2016 in Guangdong province, China. The median age of the participants was 38 years old (range from 12 to 87 years). Forty seven participants (2.47%) were men who have sex with men. Ninety five (4.99%) were screened positive for syphilis and 15(0.79%) 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 was no significant difference between age distribution and 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 two portions. The first half was used for the leukocyte esterase test at local clinics, and the other half was used for CT detection at Guangdong Provincial Dermatology Hospital. Urine samples were transported under the appropriate temperatures to the study laboratory for processing and testing. Samples for the PCR were stored at 4°C until processing.
DNA extraction and detection of CT

DNA in men urine samples were extracted and detected for CT and Neisseria gonorrhoeae (NG) by using the Roche cobas® 4800 CT/NG (Roche Diagnostics, Mannheim, Germany). The cobas 4800 system is a diagnostic assay using an automated work station to isolate nucleic acids from clinical specimens as well as a real-time instrument for the detection of CT (6). Experiments were performed under the guidance of the manufacturer’s instructions. The isolated DNA was stored at -20°C until its use for genotyping.

Amplification of ompA fragment and sequencing

The ompA genes (VS1-VS2) were amplified by PCR using a previously described method in our laboratory (Bio-Rad, T100, USA) (9,18). For the omp1 VS1–VS2 PCR, the ompA gene sequences of 11 different C. trachomatis serovars were retrieved from the GenBank. The VS1–VS2 fragment, which is known to encode type-specific epitopes, was selected as the detection target, and nested PCR primers were designed on ompA constant regions flanking variable segments VS1–VS2 with Dnastar software 5.0. 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’-ACTTTGTTTTCTGACGCGTGTG-3’ and CT4: 5’-GATTGAGCGTATTGG AAAGAAGC-3’. The fragments were 516bp and 453bp. The amplified DNA was sent to Life Technologies (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 sequence on the NCBI database by using BLAST.
**Urine leukocyte esterase test**

The leukocyte esterase test (LET) is a non-specific rapid assay used to detect the presence of an esterase enzyme produced by polymorphonuclear leucocyte in urine. LET was performed using dry chemical method at local clinics (Dirui, Changchun, China), followed by manufacturer’s instructions, before testing with PCR. Either positive or negative result was reported for every specimen.

**Statistical analysis**

SPSS version 15.0 was used for statistical analyses. Chi-square test or the Fisher’s exact test (when sample sizes were small) was used to compare the proportion between different groups. $P<0.05$ was considered to be significant.

**Results**

**Prevalence of Chlamydia infection**

Out of the 1,926 urine specimens, 1,903 (98.8%) urine samples were successfully used for valid tests of the detection of CT and NG. One hundred and sixty three (8.6%, 95%CI 3.8- 16.3%) participants were found to be positive for CT (table1) and 64 (3.36%, 95%CI 2.60- 4.27%) participants were found to be positive for NG. Among them, 14 were CT and NG co-infection cases. The highest prevalence of CT infection was observed among participants aged 21-30 (10.5%). The prevalence of CT infection in the 21-30 age group was slightly higher than 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, while different regions had different levels of CT prevalence ($\chi^2=20.80$, $P=0.00$).
**Age with genotypes**

Of the 163 urine samples that tested positive for CT by the Roche PCR assay, *ompA* gene was amplified with the nested PCR in 132 (81.0%). There were not enough amplification fragments for sequencing for the other 31 samples. Overall, 130 specimens were successfully genotyped and resulted in eight genotypes. The most prevalent genotypes were D, E, F, and J, with prevalence of 20.8%, 20.0%, 17.7%, 16.9%, respectively (table 2). Infection with K genotype was more common among people who were more than 30 years old ($\chi^2=5.48$, $P=0.02$).

**Geographic area with genotypes**

There was no significant difference between geographic area and genotypes ($\chi^2=14.68$, $P=0.40$) (table 3).

**Urine leukocyte esterase test with genotypes**

Urine leukocyte esterase test was performed for all specimens. Among 130 successfully genotyped specimens, there was no significant difference between genotype and leukocyte esterase test ($\chi^2=2.90$, $P=0.89$) (table 4).

**Discussion**

In this study, there was a high prevalence (8.6%) of CT infection from urine specimens among MSCs in Guangdong province, China. The highest prevalence was among participants aged 21-30. The most prevalent genotypes were D, E, F, and J. This study extended the existing literature by assessing CT prevalence from urine samples of MSCs, by genotyping the samples among Chinese MSCs.
The overall prevalence of CT in this study is higher compared to those described in other research in China. The prevalence of CT from urine specimens was 5.9% in Jiangsu province (15). This prevalence is also higher than the findings of a study conducted in three cities of Guangdong province in Shenzhen, Zhuhai and Qingyuan (5.5%) (18). The CT prevalence 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 among those aged 20-30 in our study. This finding is similar to the results of a study conducted in Guangdong in 2014, which indicated that men aged 25-34 have the highest incidence of CT (18). It 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 those aged 20-29 (5). Similar results were also found in Europe in 2013 (21).

The prevalence of CT infection appeared to be regionally distributed. The western and northern regions have a 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.

The most prevalent genotypes from urine specimens detected in this study were D, E, F, and J. This genotype distribution is similar to our previous study, which also indicated that E, F, J and D were the most
common genotypes among men and women patients in Guangdong province from 2005 to 2014 (9, 22). Another study conducted at Shenzhen also reported similar findings (23). It suggested that the prevalence of genotypes D, E, F, and J were stable in Guangdong province.

The most prevalent genotypes in our study were similar to those observed in the heterosexual populations of Asian and western countries. Genotype E was the most frequently identified subtype from middle-1990s to 2000 in Japan (24). Moreover, genotype E was the most prevalent (70.8%) single infection in Tunisian (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 ocular serovar and cause 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 examples of evidence for transmission of trachoma genotypes in sexual networks. Takahashi S et al isolated 11 cases of genotype B from Japanese patients (24). Similarly, two cases of genotype B were identified in 1987 and 1996 in Finland, while potential evidence of transmission of trachoma genotypes in sexual networks was identified (29).

The relationship between certain *C. trachomatis* genotypes and virulence remain weak. Though there were several reports on the possible relationship between certain genotypes and clinical features, it is not likely that typing *ompA* or *ompA* encoded protein MOMP of the urogenital strains will reveal clues to their pathogenicity (30, 31, 32). The
data indicated that there was no significant difference between genotype distribution and LET. Furthermore, we found 9 cases of genotype K among aged 30-60, but there was no genotype K among MSCs aged younger than 30. Whether genotype K is associated with age is not reported.

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

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

**Acknowledgements**

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**Declaration of conflicting interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References


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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sample size</th>
<th>No. infected</th>
<th>Infected(%) (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>93</td>
<td>8</td>
<td>8.6(3.8-16.3)</td>
</tr>
<tr>
<td>21-30</td>
<td>488</td>
<td>51</td>
<td>10.5(7.9-13.5)</td>
</tr>
<tr>
<td>31-40</td>
<td>463</td>
<td>37</td>
<td>8.0(5.7-10.9)</td>
</tr>
<tr>
<td>41-50</td>
<td>459</td>
<td>44</td>
<td>9.6(7.1-12.7)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>400</td>
<td>23</td>
<td>5.8(3.7-8.5)</td>
</tr>
<tr>
<td>total</td>
<td>1903</td>
<td>163</td>
<td>8.6(7.3-9.9)</td>
</tr>
<tr>
<td>Geographic area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearl River Delta</td>
<td>755</td>
<td>64</td>
<td>8.5(6.6-10.7)</td>
</tr>
<tr>
<td>eastern region</td>
<td>359</td>
<td>51</td>
<td>14.2(10.8-18.3)</td>
</tr>
<tr>
<td>western and northern region</td>
<td>789</td>
<td>48</td>
<td>6.1(4.5-8.0)</td>
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</table>

Table 2. Difference between age and genotypes

<table>
<thead>
<tr>
<th>Age(y)</th>
<th>B</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>J</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤30</td>
<td>1(2.1)</td>
<td>12(25.5)</td>
<td>12(25.5)</td>
<td>10(21.3)</td>
<td>5(10.6)</td>
<td>0(0)</td>
<td>7(14.9)</td>
<td>0(0)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>1(1.2)</td>
<td>15(18.1)</td>
<td>14(16.9)</td>
<td>13(15.7)</td>
<td>12(14.5)</td>
<td>4(4.8)</td>
<td>15(18.1)</td>
<td>9(10.8)</td>
</tr>
<tr>
<td>total</td>
<td>2(1.5)</td>
<td>27(20.8)</td>
<td>26(20.0)</td>
<td>23(17.7)</td>
<td>17(13.1)</td>
<td>4(3.1)</td>
<td>22(16.9)</td>
<td>9(6.9)</td>
</tr>
<tr>
<td>χ²</td>
<td>0.17</td>
<td>1.02</td>
<td>1.41</td>
<td>0.65</td>
<td>0.39</td>
<td>2.34</td>
<td>0.22</td>
<td>5.48</td>
</tr>
<tr>
<td>P</td>
<td>0.68</td>
<td>0.31</td>
<td>0.24</td>
<td>0.42</td>
<td>0.54</td>
<td>0.13</td>
<td>0.64</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table 3 Difference between geographic area with genotypes

<table>
<thead>
<tr>
<th>geographic area</th>
<th>B</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>J</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearl River Delta</td>
<td>1(2.0)</td>
<td>6(12.0)</td>
<td>9(18.0)</td>
<td>10(20.0)</td>
<td>10(20.0)</td>
<td>2(4.0)</td>
<td>11(22.0)</td>
<td>1(2.0)</td>
</tr>
<tr>
<td>eastern region</td>
<td>1(2.4)</td>
<td>10(24.4)</td>
<td>7(17.1)</td>
<td>7(17.1)</td>
<td>5(12.1)</td>
<td>1(2.4)</td>
<td>7(17.1)</td>
<td>3(7.3)</td>
</tr>
<tr>
<td>western &amp; northern regions</td>
<td>0(0.0)</td>
<td>11(28.2)</td>
<td>10(25.6)</td>
<td>6(15.4)</td>
<td>2(5.1)</td>
<td>1(2.6)</td>
<td>4(10.3)</td>
<td>5(12.8)</td>
</tr>
<tr>
<td>total</td>
<td>2(1.5)</td>
<td>27(20.7)</td>
<td>26(20.0)</td>
<td>23(17.7)</td>
<td>17(13.1)</td>
<td>4(3.1)</td>
<td>22(16.9)</td>
<td>9(7.0)</td>
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<tr>
<td>$\chi^2$</td>
<td>1.10</td>
<td>4.00</td>
<td>1.10</td>
<td>0.34</td>
<td>4.30</td>
<td>0.23</td>
<td>2.15</td>
<td>4.00</td>
</tr>
<tr>
<td>$P$</td>
<td>1.00</td>
<td>0.15</td>
<td>0.56</td>
<td>0.84</td>
<td>0.11</td>
<td>1.00</td>
<td>0.36</td>
<td>0.13</td>
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Table 4 Difference between leukocyte esterase test and genotypes

<table>
<thead>
<tr>
<th>LET</th>
<th>B</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>J</th>
<th>K</th>
<th>total</th>
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<tr>
<td>positive</td>
<td>1(50.0)</td>
<td>12(44.4)</td>
<td>13(50.0)</td>
<td>10(43.5)</td>
<td>6(35.3)</td>
<td>1(25.0)</td>
<td>12(54.6)</td>
<td>3(33.3)</td>
<td>58(44.6)</td>
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<tr>
<td>negative</td>
<td>1(50.0)</td>
<td>15(55.6)</td>
<td>13(50.0)</td>
<td>13(56.5)</td>
<td>11(64.7)</td>
<td>3(75.0)</td>
<td>10(45.4)</td>
<td>6(66.7)</td>
<td>72(55.4)</td>
</tr>
<tr>
<td>total</td>
<td>2</td>
<td>27</td>
<td>26</td>
<td>23</td>
<td>17</td>
<td>4</td>
<td>22</td>
<td>9</td>
<td>130</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>0.7</td>
<td>0.27</td>
<td>0.38</td>
<td>0.02</td>
<td>0.69</td>
<td>0.09</td>
<td>1.06</td>
<td>0.13</td>
<td>2.90</td>
</tr>
<tr>
<td>$P$</td>
<td>1</td>
<td>0.6</td>
<td>0.54</td>
<td>0.9</td>
<td>0.41</td>
<td>0.77</td>
<td>0.3</td>
<td>0.72</td>
<td>0.89</td>
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LET: leukocyte esterase test