Molecular epidemiology of Chlamydia trachomatis
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High resolution typing reveals distinct *Chlamydia trachomatis* strains in an at-risk population in Nanjing, China

Reinier J.M. Bom, Anneke van den Hoek, Qianqiu Wang, Fuquan Long, Henry J.C. de Vries, and Sylvia M. Bruisten

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**Abstract**
We investigated *Chlamydia trachomatis* strains from Nanjing, China, and whether these strains differed from Amsterdam, the Netherlands. *C. trachomatis* type was determined with multilocus sequence typing. Most strains were specific to Nanjing, but some clustered with strains from Amsterdam. This demonstrates a geographical variation in *C. trachomatis* previously left undetected.

**Introduction**
*Chlamydia trachomatis* infection is the most prevalent bacterial sexually transmitted infection (STI) worldwide. *C. trachomatis* infections are highly prevalent in China and have been on the increase over the past few decades. This has been attributed to recent political and socioeconomic developments. Molecular epidemiological studies were previously conducted in China and described that *ompA* genovars D, E, and F were the most prevalent types. This *ompA* genovar distribution is similar to distributions found elsewhere in Asia and in the rest of the world. However, these studies made use of only 1 molecular target, the *ompA* gene, which was shown recently to lack the resolution needed for molecular epidemiological studies. The use of a multilocus sequence typing (MLST) system offers the possibility of discriminating between *C. trachomatis* strains in greater detail.

In the present study, a MLST method was applied, which was designed to differentiate *C. trachomatis* strains at a population level. The samples were derived from patients visiting a large STI clinic in Nanjing, China. We investigated to which degree *C. trachomatis* strains found among heterosexuals from Nanjing differed from strains found among heterosexuals in the city of Amsterdam, the Netherlands, which are geographically very distant. The Dutch samples were collected among heterosexuals at the STI clinic, as described in a previous study.

**Methods and materials**
The study was conducted among visitors of the Institute of Dermatology’s STI Clinic at the National Center for STI Control in Nanjing, China. The recruitment period ran from January 2010 through February 2010 (pilot study) and from November 2010 through September 2011. All *C. trachomatis*–infected visitors were eligible for inclusion. The comparison group consisted of heterosexual participants who visited the STI outpatient clinic of the Public Health Service in Amsterdam, the Netherlands, between November 2009 and May 2010. The Amsterdam participants have been
described in a previous study. Differences in demographic data between participants from the 2 countries were tested using Pearson \( \chi^2 \) test for categorical data and Mann-Whitney U test for continuous data. Analyses were performed with SPSS 19 (SPSS Inc, Chicago, IL).

Visitors were routinely tested for STI according to standard procedures of the Nanjing STI Clinic. Dacron-tipped swab samples (Alere Medical, Shanghai, China) and dry ProbeTec swab samples (Becton, Dickinson and Company, Breda, the Netherlands) were taken consecutively from the vagina or urethra. The Dacron-tipped swab samples were tested for the presence of \( C. \) trachomatis, using the Clearview \( Chlamydia \) MF assay (Alere Medical). In case of a positive test result, the ProbeTec swab was sent to the Public Health Laboratory in Amsterdam, the Netherlands. Demographic data were obtained through structured questionnaires conducted by health care workers in Nanjing STI Clinic.

The ProbeTec swabs were eluted in 500 µL phosphate-buffered saline, in which the nucleic acids were extracted by isopropanol precipitation and tested for the presence of genomic \( C. \) trachomatis DNA. DNA isolates were amplified by a nested polymerase chain reaction and sequenced for the regions \( \text{ompA, CT046 (hctB), CT058, CT144, CT172, and CT682 (pbpB).}^{12,14} \)

The cleaned primer-to-primer sequences were checked against the \( C. \) trachomatis MLST database (mlstdb.bmc.uu.se). Samples were only included in the analyses when all alleles were successfully amplified, sequenced, and identified and therefore had obtained a full MLST profile. A minimum spanning tree was generated using MLST profiles. Cluster analysis was performed allowing single-locus variance through use of BioNumerics 7 (Applied Maths, Sint-Martens-Latem, Belgium). A cluster was defined as a group of sequence types (STs) differing by not more than 1 locus from another ST within that group (single-locus variance) and had to include at least 5% of the total number of samples. The identified \( C. \) trachomatis clusters were compared with the Dutch samples obtained from heterosexual visitors of the STI outpatient clinic in Amsterdam.

**Results**

During the study period, 59 men and 42 women were enrolled in Nanjing, contributing 101 samples. In 91 samples (90%), there was enough \( C. \) trachomatis DNA for genotyping by MLST. For 1 sample, 2 sequence variants were detected in the fluorescent chromatograms of all amplified regions. We assumed that 2 strains were present in this sample, but because the individual MLST profiles could not be established, this sample was excluded. The remaining 90 samples were derived from 58 men and 32 women. Participant characteristics are shown in Table 1. The median age of the Chinese participants was 35 years. Most (67%; \( n = 58 \)) of these participants were married or in a steady relationship. The median number of sexual partners in the previous 6 months was 2. A total of 79% \( (n = 46) \) of the men reported having paid for sex with women.
in the previous 6 months, and 28% (n = 8) of the women reported having received money for sex in the previous 6 months. One man reported having had sex with another man in the previous 6 months. Significant demographical differences were seen between the Nanjing and Amsterdam populations (Table 1).

Because ompA is part of the MLST scheme, genovars could be assigned to all typed samples. Among the participants in the Nanjing area, we found 13 different ompA variants, belonging to 9 different genovars. The most common types were F (33%; n = 30), E (17%; n = 15), D (14%; n = 13), and J (13%; n = 12). The other types were genovar G (9%; n = 8), K (9%; n = 8), H (2%; n = 2), B (1%; n = 1), and I (1%; n = 1). Using all 6 loci from the MLST scheme, 34 different C. trachomatis STs could be determined, of which 24 were new to the publicly available C. trachomatis MLST database at the time of writing (mlstdb.bmc.uu.se). The number of samples per ST ranged from 1 to 19. In the minimum spanning tree generated for these 90 samples, 5 clusters could be distinguished (Figure 1). Cluster 1 (n = 25) contained most of the genovar F samples, whereas cluster 2 (n = 15) and cluster 3 (n = 12) consisted of genovar E and J samples, respectively. Cluster 4 (n = 11) and cluster 5 (n = 6) contained most of the genovar D and K samples. There were also

<table>
<thead>
<tr>
<th></th>
<th>Nanjing</th>
<th>Amsterdam</th>
<th>P</th>
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<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (64)</td>
<td>86 (34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>32 (36)</td>
<td>170 (66)</td>
<td></td>
</tr>
<tr>
<td>Age, y*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>35 (30-42)</td>
<td>23 (21–27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. sexual partners in the past 6 mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2 (1–2)</td>
<td>2 (1–4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Paid or received money for sex in the past 6 mo*, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54 (62)</td>
<td>6 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>33 (38)</td>
<td>250 (98)</td>
<td></td>
</tr>
</tbody>
</table>

* Data were missing for 3 participants from Nanjing. IQR indicates interquartile range.
samples from 3 sexual couples, and within each couple, the 2 partners had identical sequences for all 6 loci.

We also obtained a minimum spanning tree using 90 samples from Nanjing, China, and 256 reference samples from Amsterdam, the Netherlands, and found large differences in the distribution of samples from the 2 cities (Figure 2). Clusters 1, 3, and 5 predominantly or fully comprised samples from Nanjing, whereas clusters 6, 8, 9, and 10 almost exclusively contained samples from Amsterdam. There were mixed clusters (clusters 2, 4, and 7) that contained *C. trachomatis* strains from both Nanjing and Amsterdam.

**Discussion**

As in previous studies, we found that genovars D, E, and F were the most prevalent *ompA* genovars among *C. trachomatis*-infected participants in Nanjing, China. Although this genovar distribution did not differ between
Chinese and Dutch samples, clusters of C. trachomatis strains associated with both countries were largely separated using high-resolution MLST. This demonstrates that a geographical variation in circulating C. trachomatis strains does exist and that previous studies using ompA genovar typing failed to detect this variation because of the low resolution of the typing method. Interestingly, although most MLST genotypes were unique to Nanjing, a few were identical to strains circulating in Amsterdam. Especially the large genovar E ST seems to be prevalent in both countries. Because sexual mixing between partners from the 2 places is unlikely, the occurrence of identical strains at 2 geographically distant locations shows the genomic stability of some C. trachomatis strains over a long period.

Because there were demographic differences between the participants from Nanjing and Amsterdam, the differences in genotype distribution may not be exclusively explained by geographical variation. However, it seems unlikely that these demographical differences alone could result in the observed variation. In addition, this study was conducted at a single clinic in Nanjing, and therefore, this study may not be representative for the distribution of C. trachomatis strains in the whole of China. Multicenter studies with inclusion sites across China could reveal a comprehensive picture of the strains circulating in China at large. A similar study on the prevalence of C. trachomatis was performed a decade ago. Also, a limitation is the use of the Clearview Chlamydia MF assay as the method of screening for C. trachomatis infections in Nanjing. Because this assay has a described low sensitivity, the distribution of C. trachomatis types might be biased toward strains with a higher bacterial load. Previous studies using genovar typing, however, found no associations between

Figure 2. Minimum spanning tree of 90 C. trachomatis-positive samples from the Nanjing Area, 2010 to 2011, and 256 reference samples from Amsterdam Area, 2009 to 2010. Sizes of the node discs are proportional to the number of samples of each ST; branches show 1 locus difference; halos indicate clusters; and colors indicate city of sampling; Red: Nanjing (n = 90). Blue: Amsterdam (n = 256).
An assumed higher bacterial load has probably positively influenced the sensitivity of the use of the collected dry swabs for MLST.

The findings of the distinct C. trachomatis cluster distribution and geographical variation for Nanjing, China, and Amsterdam, the Netherlands, need to be confirmed in a global setting by MLST typing and cluster analysis of C. trachomatis samples. This work was recently initiated through the publicly available C. trachomatis MLST database (mlstdb.bmc.uu.se), which includes MLST studies from various countries and risk groups. Enlarging this database will increase our knowledge on the worldwide distribution of C. trachomatis and uncover the effects of sexual mixing in a globalizing world, thus contributing to improved screening and prevention programs in the future.

REFERENCES


