Molecular epidemiological research of *Treponema pallidum* infection in Haikou

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**Abstract**

**Objective:** To discuss genotypes distribution and molecular epidemiological characters of *Treponema pallidum* (*T. pallidum*) infection in Haikou. **Methods:** A total of 102 specimens of external genitals ulcer from patients with suspected syphilitic chancre who visited from March 2012 to February 2013 were collected and detected by dark-field microscope and PCR method. Acidic repeat protein (arp) gene of *T. pallidum* and *T. pallidum* repeat (tpr) gene families were amplified in PCR-positive samples by nested PCR. The number of repeats presented in the arp gene and the restriction fragment length polymorphism by Mse I in the tpr gene were analyzed by electrophoresis. **Results:** Out of 102 patients with suspected syphilitic chancre, 55 cases (53.2%) were positive by dark-field microscopy and 89 cases (87.3%) by *T. pallidum* bmp PCR. Seven genotypes were found in 72 cases, including 14d (28, 38.9%), 12d (12, 16.7%), 13d (11, 15.3%), 14b (9, 12.5%), 14a (5, 6.9%), 12a (3, 4.2%), 15d (2, 2.8%) 10d (1, 1.4%), 12i (1, 1.4%). **Conclusions:** Genotype 14 d is the predominant type of *T. pallidum* in Haikou and bmp PCR has high sensitivity.

**1. Introduction**

Syphilis is one of sexually transmitted diseases. It can be transmitted by sexual contact and by mother-infant vertical transmission. The pathogen is *Treponema pallidum* (*T. pallidum*) (*Tp*), which can result in multisystem injury by invading nerve, skeleton, skin, mucous membrane, angiocarpy etc. and can result in abortion, stillbirth and congenital syphilitic fetus. Besides, syphilis has synergistic action with HIV and can promote HIV infection. Recently, the epidemic situation of syphilis is significantly aggravated. With incidence as the top 5 in Hainan, and also the top in nation, it has become the main infectious disease leading to damage to public health[1]. Therefore, syphilis is one of important public health problems in Hainan. Genotyping system of *Tp* is important precondition for molecular epidemiological research on syphilis, and has been the key project in the world for many years. It is reported that some researchers tried genotyping by many methods such as DNA hybridization, single nucleotide polymorphism, but all failed[2]. Until the end of last century, a new and simple genotyping system has been put forward by Pillay et al from CDN, which initiates new stage molecular epidemiological researches.

**2. Materials and methods**

**2.1. Research objects**

Specimens of external genitals ulcer from 102 patients with suspected syphilitic chancre who visited from March 2012 to February 2013 were selected, including 77 males and 25 females, aged 17-48 years old.
2.2. Specimen collection

Cotton swab with saline dip was used to clear the surface external genitals ulcer. The surrounding skin was pinched to exude tissue liquid. The exuded liquid was scraped by dull knife to make smear for dark-field microscope. The liquid around base of ulcer was fetched by cotton swab, eluted in 1-2 mL saline, and centrifuged at 13 000 r/min for 10 min. The supernatant was abandoned, and the sediment was restored in refrigerator at -70 °C.

2.3. Experimental materials

Two pairs of primers (nested primer) were designed based on Tp basic membrane protein (bmp). The amplified fragment length of outer primer was 620 bp, and the length of inner primer was 506 bp. The primer sequences were as follows:

Outer primer 5’-CTCAGCACTGCTGAGCGTAG-3’; 5’-AACGCCTCCATCGTCAGACC-3’;

Inner primer 5’-CAGGTAACGGATGCTGAAGT-3’; 5’-CGTGGCAGTAAACCGCAGTCT-3’.

All primers were designed by Sangon Bioengineering Technology Limited Company in Shanghai. The reaction system and amplification condition were based on reference[4,5].

2.4. Genotyping

Tp bmp with PCR positive result was genotyped.

2.4.1. Genotyping of acidic repeat protein gene (arp)

Conserved sequence across the gene was selected, and modified half nested PCR method by Zheng was used, which included upstream of APRI outer primer (5-CGTGCGCGGGTGGTCTCAAAC-3), upstream of ARPI inner primer (5-CAAGTCAGGAACGGCTGTCCCTTGC-3) and downstream of ARP2a (5-GGTATCACCTGGGGATGCGCACG-3). Amplification condition was according to Fanella et al and Harper et al[5,6]. After gel electrophoresis, fragment length of amplification product was analyzed by gel-image analysis.

3.3. tpr gene analysis

Out of 89 cases with positive result of Tp screening, 78 cases were positive in arp gene detection. 14 type was the main type (n=42, 53.8%), followed by 12 type (n=18, 23.1%), 13 type (n=15, 19.2%), 15 type (n=2, 3.8%) and 10 type (n=1, 1.3%) (Figure 2).

3.2. PCR result

PCR result showed that 89 cases had amplified bmp gene, and the positive rate was 87.3% (Figure 1).

Figure 1. Amplification of Tp bmp gene.
M: Standard control; 1 Tp Nichol strain; 2-5 Clinical specimen.

Figure 2. arp genotyping of some clinical specimens.
M: standard control; 1: Tp Nichol strain; 2-5 Clinical specimen.

3. Results

3.1. Dark-field microscope result

The microscope result showed that out of 102 cases, 55 cases had Tp infection (53.9%).

3.2. PCR result

PCR result showed that 89 cases had amplified bmp gene, and the positive rate was 87.3% (Figure 1).

Restricted fragment length polymorphisms method was used. Primer sequence of tpr gene (outer primer B1: 5-ACTGCGCTCGCTCCACACTTG-3, B2: 5-CCTACAGGAAGGTTGAC-3; inner primer F1: 5-CAGGTGTTTGCCTGTAAGC-3, F2: 5-AATCAGGGAGTAAACCGTC-3), amplification condition was according to Fanella et al and Harper et al[5,6]. Amplification product was digested by Mse I, and restriction map was analyzed after 3% agarose gel electrophoresis.
3.4. tpr gene analysis

Out of 89 cases with positive result of Tp screening, 82 cases were positive in tpr detection. MseI restriction map showed d type was the main type (n=58, 70.7%), followed by b type (n=11, 13.4%), a type (n=9, 11.0%), I type (n=3) and g type (n=1) (Figure 3).

![Figure 3. tpr genotyping of some clinical specimens.](image)

M: standard control; 1: Tp Nichol strain, a type; 2: d type; 3: i type; 4-7: d type; 8: b type.

3.5. Molecule subtypes of Tp

There were 72 cases with positive result both in arp and tpr gene analysis. The molecule subtypes of Tp were listed in Table 1.

<table>
<thead>
<tr>
<th>Tp subtypes</th>
<th>10d</th>
<th>12a</th>
<th>12d</th>
<th>12i</th>
<th>13d</th>
<th>14a</th>
<th>14b</th>
<th>14d</th>
<th>15d</th>
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<td>12</td>
<td>1</td>
<td>11</td>
<td>5</td>
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</tr>
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<td>Percentage (%)</td>
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<td>4.2</td>
<td>16.7</td>
<td>1.4</td>
<td>15.3</td>
<td>6.9</td>
<td>12.5</td>
<td>38.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

4. Discussion

Dark-field microscope is the main detection method of syphilis. But the application is limited by low sensitivity and high technology requirement(7,8). In our study, we detected specimens from 102 cases with suspected syphilitic chancre by dark-field microscope, and the detection rate is 53.9%. At the same time, nested-PCR was used to amplify base membrane protein gene bmp, and the detection rate is 87.3%, with the significantly increased sensitivity. All 55 specimens which were positive in dark-field microscope observation, also shows positive result in nest-PCR detection. arp of different Tp strains contains 60 repeated sequences with base pairs in different length. After PCR and digestion by Mse I, tpr shows restricted fragment length polymorphisms. Based on these different characters of arp and tpr in different Tp strains, Pillay et al combined these two methods, and established more simple and more reliable method for Tp genotyping(4). In high risk areas in United States and South African, this method has been applied in molecular epidemic research of syphilis. In our study, based on this method and modified method by Deng et al, we amplified base membrane protein gene bmp by nested-PCR to screen suspected specimens, then differentiate subtypes in positive specimens. A total of 9 subtypes are obtained, and 14 type is the main type (38.9%), which is similar to other researches(9-11). However, due to the source of clinical specimens and lack of residents differentiation, it still need further study whether 14d is the dominant import epidemic strain in Haikou or in other districts of Hainan Province, and whether it is more likely transmitted and more toxic. Besides, considering the limited number of minority subjects (n=2), it is also need further study on diversity of Tp molecular subtype in other nationalities of Hainan.

References