Evaluation of Macrolide Resistance and Enhanced Molecular Typing of *Treponema pallidum* in Patients with Syphilis in Taiwan: a Prospective Multicenter Study

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Studies of macrolide resistance mutations and molecular typing using the newly proposed enhanced typing system for *Treponema pallidum* isolates obtained from HIV-infected patients in the Asia-Pacific region are scarce. Between September 2009 and December 2011, we conducted a survey to detect *T. pallidum* using a PCR assay using clinical specimens from patients with syphilis at six major designated hospitals for HIV care in Taiwan. The *T. pallidum* strains were genotyped by following the enhanced molecular typing methodology, which analyzed the number of 60-bp repeats in the acidic repeat protein (arp) gene, *T. pallidum* repeat (tpr) polymorphism, and the sequence of base pairs 131 to 215 in the *tp0548* open reading frame of *T. pallidum*. Detection of A2058G and A2059G point mutations in the *T. pallidum* 23S rRNA was performed with the use of restriction fragment length polymorphism (RFLP). During the 2-year study period, 211 clinical specimens were obtained from 136 patients with syphilis. *T. pallidum* DNA was isolated from 105 (49.8%) of the specimens, with swab specimens obtained from chancres having the highest yield rate (63.2%), followed by plasma (49.4%), serum (35.7%), and cerebrospinal fluid or vitreous fluid (18.2%) specimens. Among the 40 fully typed specimens, 11 subtypes of *T. pallidum* were identified. Subtype 14f/f (18 isolates) was the most common isolates, followed by 14f/c (3), 14b/c (3), and 14k/f (3). Among the isolates examined for macrolide resistance, none had the A2058G or A2059G mutation. In conclusion, we found that type 14f/f was the most common *T. pallidum* strain in this multicenter study on syphilis in Taiwan and that none of the isolates exhibited 23S rRNA mutations causing resistance to macrolides.

Syphilis is a multistage, sexually transmitted disease, caused by the spirochete *Treponema pallidum*, which may lead to severe complications such as neurosyphilis, cardiovascular syphilis, and adverse pregnancy outcomes if left untreated. Furthermore, syphilis has also been documented to facilitate both infectivity of and susceptibility to HIV infection (10, 28, 33). The incidence of syphilis is rising again in several developed countries since 2000, after a steady decline over the past 60 years following the introduction of penicillin (11, 33). In the United States, the rate of primary and secondary syphilis reported increased annually after 2001 and reached 4.6 cases per 100,000 population in 2009 (4); overall increases in rates have been observed primarily among men (increasing from 3.0 cases per 100,000 population in 2001 to 7.9 cases per 100,000 population in 2010) (4). In addition, outbreaks of syphilis have been noted among men who have sex with men (MSM) in many cities in the United States, Europe, and Australia (5, 12, 29). In Taiwan, 6,482 cases of syphilis (diagnosed by elevated titers of rapid plasma reagin [RPR] or the Venereal Disease Research Laboratory [VDRL] test and positivity for *Treponema pallidum* hemagglutination [TPHA]) were reported to the Taiwan CDC in 2010, which resulted in an annual incidence of 28.0 per 100,000 population (3). Under such circumstances, early diagnosis of syphilis, effective treatment of patients with the early stage of syphilis, and detection of outbreaks are essential components for disease control.

Because the causative agent, *T. pallidum*, cannot be cultivated on standard bacteriological media, the diagnosis of syphilis is usually made by either detection of spirochetes by dark-field microscopy or immunostaining with fluorescent-antibody-conjugated reagents of clinical specimens or by serology. PCR methods to detect *T. pallidum* have been developed since 1990 for the purpose of improving the sensitivity and specificity of laboratory diagnosis. A recently developed assay in which primers are rationally designed based on two unique characteristics of the DNA polymerase I gene of *T. pallidum* can detect pathogenic *T. pallidum* subspecies in clinical specimens with a sensitivity of 95.8% and a specificity of 95.7% (14). Moreover, with the use of PCR assays, researchers are able to perform molecular typing and detect macrolide resistance of *T. pallidum*.

A molecular subtyping scheme for *T. pallidum* was first developed by Pillay et al. in 1998 using the number of 60-bp tandem repeats (13). This scheme can detect pathogenic *T. pallidum* subspecies in clinical specimens with a sensitivity of 95.8% and a specificity of 95.7% (14). In this scheme, the distribution of a number of 60-bp tandem repeat length polymorphism (RFLP) is used in a PCR assay. In this study, we conducted a survey to detect *T. pallidum* using a PCR assay using clinical specimens from patients with syphilis at six major designated hospitals for HIV care in Taiwan. The *T. pallidum* strains were genotyped by following the enhanced molecular typing methodology, which analyzed the number of 60-bp repeats in the acidic repeat protein (arp) gene, *T. pallidum* repeat (tpr) polymorphism, and the sequence of base pairs 131 to 215 in the *tp0548* open reading frame of *T. pallidum*. Detection of A2058G and A2059G point mutations in the *T. pallidum* 23S rRNA was performed with the use of restriction fragment length polymorphism (RFLP). During the 2-year study period, 211 clinical specimens were obtained from 136 patients with syphilis. *T. pallidum* DNA was isolated from 105 (49.8%) of the specimens, with swab specimens obtained from chancres having the highest yield rate (63.2%), followed by plasma (49.4%), serum (35.7%), and cerebrospinal fluid or vitreous fluid (18.2%) specimens. Among the 40 fully typed specimens, 11 subtypes of *T. pallidum* were identified. Subtype 14f/f (18 isolates) was the most common isolates, followed by 14f/c (3), 14b/c (3), and 14k/f (3). Among the isolates examined for macrolide resistance, none had the A2058G or A2059G mutation. In conclusion, we found that type 14f/f was the most common *T. pallidum* strain in this multicenter study on syphilis in Taiwan and that none of the isolates exhibited 23S rRNA mutations causing resistance to macrolides.
repeats in the acidic repeat protein (arp) gene and restriction fragment length polymorphism (RFLP) analysis of the T. pallidum repeat (tpr) gene (25). With the use of this method, several studies have described the most common subtypes in different countries: 14d in the United Kingdom, Canada, South Africa, China, and Madagascar; 14f in the United States; and 14a in Portugal (7, 9, 13, 17–19, 26, 27, 30). However, its inadequate power to discriminate the most common subtypes limited the use of this typing method in outbreak investigations. Given this limitation, Marr and colleagues developed an enhanced molecular typing method to improve discrimination by analyzing a short region of the tp0548 gene in addition to analysis of the arp and tpr genes (17).

Studies on macrolide resistance mutations and molecular types of isolates of T. pallidum obtained from MSM in the Asia-Pacific region using the newly proposed method are limited (18, 24, 34). In this study, we aimed to investigate the molecular epidemiology and prevalence of azithromycin-resistant T. pallidum among patients with early syphilis in Taiwan.

(Preliminary analyses of these data were presented at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 17 to 20 September 2011 [11a].)

MATERIALS AND METHODS

Setting and study population. This was a multicenter, prospective survey conducted at the infectious diseases clinics of six major hospitals designated for HIV care between September 2009 and December 2011; two of these hospitals are located in northern Taiwan, while the other four hospitals are in southern Taiwan. Adult patients who presented with primary, secondary, or early latent syphilis, neurosyphilis, and uveitis with a rapid plasma reagin (RPR) titer of ≥1:4 and positive Treponema pallidum particle hemagglutination (TPHA) assay results were enrolled. Patients who had received antibiotics for syphilis before enrollment were excluded. Data collected included the patient’s demographics, risk for HIV infection, HIV serostatus, CD4 count and plasma HIV RNA load (for HIV-infected patients), coinfection, stage of syphilis, treatment of syphilis, HIV serostatus, and plasma HIV RNA load (for HIV-infected patients).

Collection of clinical specimens. All appropriate clinical specimens were collected before antibiotics were administered. Specimens of ulcer lesions were collected by swabs that were pressed and rolled over the ulcers. The exudate expressed on the lesion was absorbed onto Dacron swabs (CultureSwab EZ; BD, Franklin Lakes, NJ). Blood specimens were obtained from patients with primary, secondary, and early latent syphilis. The white–blood specimens from the two hospitals in northern Taiwan were collected in EDTA-coated containers (BD Vacutainer or BD Hemogard) and centrifuged within 1 day. The cerebrospinal fluid (CSF) and vitreous fluid specimens were collected in sterile containers. Multiple specimens were obtained from patients with multiple ulcers or concurrent primary and secondary lesions. All specimens from northern Taiwan were transported to the central laboratory at the National Taiwan University Hospital within 1 day. The blood specimens from the other four hospitals in southern Taiwan were collected in serum containers (BD Vacutainer SST tubes) and transported at 4°C to the laboratory within 2 days.

Laboratory investigations. Treponemal DNA was extracted from clinical specimens using the Qiagen DNA minikit (Qiagen, GmbH, Germany) according to the manufacturer’s instructions. PCR assay was performed to detect the presence of T. pallidum by amplifying a 378-bp fragment of the T. pallidum polymerase A gene (polA) as described by Liu et al. (14). RFLP of the 23S rRNA was performed to detect macrolide resistance as described by Lukehart et al. (15). Because azithromycin treatment failure was reported in a case of syphilis in which T. pallidum harbored an A2059G point mutation (20), we also investigated the presence of this point mutation in archived specimens.

PCR amplification of a 60-bp tandem repeat region within the arp gene was carried out in a final volume of 50 μl containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM deoxyribonucleoside triphosphates (dNTPs), 0.3 μM each primer (Arp1A/Arp2A), 2.5 U platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 10 μl DNA template. The primer pair used was Arp1A (5′-CAA GTG ACC GGG CAC GTC CCC TTG C-3′) and Arp2A (5′-GGT ATC ACC TGG TGG GGA TGC GCA CG-3′). The amplification conditions were 94°C for 5 min, 45 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 2.5 min, and a final extension at 72°C for 10 min. The number of arp gene repeats was determined by comparing the sizes of the PCR amplicons with those of the Nichols strain of T. pallidum, which has 14 repeats.

A nested PCR assay was used to amplify the tpr gene. The first primer pair used was B1 (5′-ACT GGC TCT GCC ACA GTT GA-3′) and A2 (5′-CTA CCA GGA GAG GGT GAC GC-3′). The amplification conditions were 94°C for 5 min, 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2.5 min, and a final extension at 72°C for 10 min. A 1-μl aliquot of the first-round PCR product was used for the second-round PCR, for which the conditions were the same as in the first round except that annealing was done at 59°C. The second primer pair used was Tpr-F (5′-CAG GGT TTG CCG TTA AGC-3′) and Tpr-R (5′-AAT CAA GGG AGA ATA CCG TC-3′). The tpr amplicons were digested with MseI, and the digestion patterns were compared with published data (25).

One sense primer, Tp0548-F (5′-GGT CCC TAT GAT ATC GTG TTC G-3′), and two antisense primers, Tp0548-R1 (5′-GGT TTC GGT TTG TGA GTC AT-3′) and Tp0548-R2 (5′-GTC ATG CTG CTA CGA TTG G-3′), were used to amplify DNA from blood specimen (Tp0548-F and Tp0548-R1) and lesion exudates (Tp0548-F and Tp0548-R2). The amplification conditions were 94°C for 2 min, 40 cycles of 95°C for 1 min, 62°C for 70 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. The amplicons were sequenced for genotyping (17).

Statistical analysis. Statistical Package for the Social Sciences (SPSS) statistics 17.0 software (IBM Corporation, Somers, NY) was used for statistical analysis. Associations between categorical variables were determined by the χ2 test or the Fisher exact test. The Student t test was used to determine differences between continuous variables. Results with P values of <0.05 were considered to be statistically significant.

RESULTS

During the 2-year study period, a total of 211 specimens were collected from 136 patients. The demographic data and clinical presentations of the patients enrolled are shown in Table 1. Of the 132 patients with available data, all were male, with an age ranging from 19.6 to 57.9 years (mean, 30.1 years), and 93 (70.5%) were HIV infected. Of the 116 patients who reported sexual orientation, 109 (94.0%) were MSM. Of the 132 patients with reported stages of syphilis, 38 (28.8%) were diagnosed with primary syphilis, 81 (61.4%) with secondary syphilis, 17 (12.9%) with concurrent primary and secondary syphilis, 26 (19.7%) with early latent syphilis, 4 (3.0%) with neurosyphilis and syphilitic uveitis, and 1 (0.8%) with concurrent primary and secondary syphilis, neurosyphilis, and syphilitic uveitis. Only one patient reported use of a macrolide antibiotic within 1 year prior to the diagnosis of syphilis. Compared with HIV-uninfected patients, HIV-infected patients had a significantly higher proportion of concurrent primary and secondary syphilis (62.0 versus 15.0%; P = 0.001).

Each patient provided one to six clinical specimens (mean, 1; median, 1.5). Of the 211 specimens, 125 (59.2%) were blood samples, 75 (35.5%) swab samples, 9 (4.3%) CSF, and 2 (0.9%) vitreous...
identified their sexual orientation.

Stage of syphilis, HIV infection, RPR titer, mean (SD) log2
6.3 (1.7) 6.3 (1.7) 6.3 (1.8) >0.99

Sixty-four and 52 patients testing positive and negative in the PCR assay, respectively, of specimens that were positive for the polA and 23S rRNA PCR, respectively. Of specimens that were positive or negative by the screening PCR assay vs. 7.7%; those from patients with primary or latent syphilis (58.6% significantly higher yield rate in screening PCR of blood samples versus 64.3%; P > 0.99) and swab samples (65.2% versus 66.7%; P > 0.99) between HIV-infected and HIV-uninfected patients.

All typeable molecular types were consistent in each patient who provided more than one specimen. Of the 105 amplifiable specimens, 40 (38.1%) tested positive for all tpr, arp, and tp0548 genes to produce 11 full subtypes. The distribution of the typeable specimens is shown in Fig. 2. Of the 36 nonduplicated, fully typeable strains of T. pallidum, 14f/f was the most common subtype (18/36; 50%), followed by 14f/c, 14b/c, and 14k/f (all 3/36; 8.3%). Comparisons of clinical characteristics between patients infected with 14f/f and those infected with non-14f/f subtypes are shown in Table 2. Patients infected with subtype 14f/f were more likely to present with primary syphilis. In patients with secondary syphilis, more patients infected with the 14f/f subtype developed a Jarisch-Herxheimer reaction following benzathine penicillin treatment (5/6 [83.3%] versus 2/9 [22.2%]; P = 0.04) (data not shown). Of all specimens with amplifiable 23S rRNA by PCR, none were detected to have an A2058G or A2059G mutation, which confers resistance to macrolides.

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### Table 1: Clinical characteristics of patients with syphilis who tested positive or negative by the screening PCR assay

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n = 132)</th>
<th>Patients testing positive (n = 73)</th>
<th>Patients testing negative (n = 59)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD), yr</td>
<td>31.0 (7.5)</td>
<td>30.4 (7.0)</td>
<td>31.8 (8.2)</td>
<td>0.29</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>132 (100)</td>
<td>73 (100)</td>
<td>59 (100)</td>
<td></td>
</tr>
<tr>
<td>MSM, n (%)a</td>
<td>109 (94.0)</td>
<td>61 (95.3)</td>
<td>48 (92.3)</td>
<td>0.70</td>
</tr>
<tr>
<td>RPR titer, mean (SD) log2</td>
<td>6.3 (1.7)</td>
<td>6.3 (1.7)</td>
<td>6.3 (1.8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>HIV infection, n (%)</td>
<td>93 (70.5)</td>
<td>51 (69.9)</td>
<td>42 (71.2)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

* Sixty-four and 52 patients testing positive and negative in the PCR assay, respectively, identified their sexual orientation.

### Table 2: Comparisons of demographic and clinical characteristics between patients infected with 14f/f and non-14f/f subtypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>14f/f (n = 18)a</th>
<th>Non-14f/f (n = 18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD), yr</td>
<td>31.2 (5.8)</td>
<td>30.4 (6.4)</td>
<td>0.70</td>
</tr>
<tr>
<td>MSM, n (%)b</td>
<td>12 (92.3)</td>
<td>15 (100)</td>
<td>0.46</td>
</tr>
<tr>
<td>RPR titer, mean (SD) log2</td>
<td>6.1 (1.9)</td>
<td>6.1 (1.7)</td>
<td>0.98</td>
</tr>
<tr>
<td>HIV infection, n (%)c</td>
<td>13 (81.3)</td>
<td>15 (83.3)</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

* For reported stage of syphilis, n = 16.

b Thirteen and 15 patients infected with 14f/f and non-14f/f subtypes, respectively, identified their sexual orientation.

c Sixteen and 15 patients infected with 14f/f and non-14f/f subtypes, respectively, had available information on HIV serostatus.
TABLE 3 Summary of the published studies that used enhanced molecular typing and conventional molecular typing of clinical strains of *Treponema pallidum*

<table>
<thead>
<tr>
<th>Study site(s)</th>
<th>Study yr</th>
<th>MSM (%)</th>
<th>HIV infection (%)</th>
<th>Stage(s) of syphilis</th>
<th>Type(s) of specimen</th>
<th>Screening PCR assay</th>
<th>Positive rate (%) of screening assay</th>
<th>Yield rate (%) for arp, tpr, tpp65, respectively&lt;sup&gt;d&lt;/sup&gt;</th>
<th>No. of specimens typed/identified</th>
<th>Most common subtype (%)</th>
<th>A2058G mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced</td>
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</tr>
<tr>
<td>Muss et al. (17)</td>
<td>U.S., China, Ireland, Madagascar, China, Taiwan</td>
<td>2005-2008</td>
<td>93.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Primary, secondary, early latent, CNS&lt;sup&gt;b&lt;/sup&gt;, uveitis</td>
<td>Blood, lesion exudate, CSF</td>
<td>polA</td>
<td>67.3</td>
<td>91.3, 90.4, 95.0</td>
<td>197/27</td>
<td>14d (50.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Peng et al. (24)</td>
<td>China</td>
<td>2008-2010</td>
<td>90.0</td>
<td>Primary, secondary</td>
<td>Plasma, serum, primary ulcer, CSF, vitreous fluid</td>
<td>tpp47</td>
<td>NA</td>
<td>NA</td>
<td>16d (50.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Wu et al. (present report)</td>
<td>Taiwan</td>
<td>2009-2011</td>
<td>85.0</td>
<td>Primary, secondary, early latent</td>
<td>Blood, lesion exudate, CSF</td>
<td>polA</td>
<td>49.8</td>
<td>95.0</td>
<td>40/11</td>
<td>14f (50.0)</td>
<td>0 (0)</td>
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<tr>
<td>Conventional</td>
<td></td>
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<tr>
<td>Pillay et al. (25)</td>
<td>U.S., Madagascar, South Africa</td>
<td>2007-2009</td>
<td>NA</td>
<td>Primary, secondary, congenital</td>
<td>Primary ulcer, secondary lesion, whole blood, plasma, serum, vitreous fluid</td>
<td>tpp47, polA</td>
<td>49/449 (10.9)</td>
<td>100.0, 83.7 —</td>
<td>36/4</td>
<td>14d (39.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Sutton et al. (30)</td>
<td>U.S.</td>
<td>1998-1999</td>
<td>0</td>
<td>Primary, secondary, latent</td>
<td>Primary ulcer, whole blood</td>
<td>polA</td>
<td>56/112 (50.0)</td>
<td>NA</td>
<td>45/10</td>
<td>14f (53.3)</td>
<td>NA</td>
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<tr>
<td>Pope et al. (27)</td>
<td>U.S.</td>
<td>1999-2003</td>
<td>NA</td>
<td>Primary, secondary</td>
<td>Primary ulcer, secondary lesion</td>
<td>polA</td>
<td>27/61 (44.3)</td>
<td>NA</td>
<td>23/7</td>
<td>14f (52.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Katz et al. (13)</td>
<td>U.S.</td>
<td>2004-2007</td>
<td>84.3</td>
<td>Primary, secondary</td>
<td>Primary ulcer, secondary lesion</td>
<td>polA</td>
<td>71/74 (95.9)</td>
<td>97.2, 98.6, —</td>
<td>69/8</td>
<td>14d (75.4)</td>
<td>42.82 (67.7)</td>
</tr>
<tr>
<td>Martin et al. (19)</td>
<td>Canada</td>
<td>2007-2009</td>
<td>NA</td>
<td>Primary, secondary, congenital</td>
<td>Primary ulcer</td>
<td>polA</td>
<td>49/449 (10.9)</td>
<td>100.0, 83.7 —</td>
<td>36/4</td>
<td>14d (83.3)</td>
<td>7/4 (16.3)</td>
</tr>
<tr>
<td>Cruz et al. (8)</td>
<td>Colombia</td>
<td>2003-2009</td>
<td>NA</td>
<td>Secondary</td>
<td>Secondary ulcer, whole blood</td>
<td>tpp47, polA</td>
<td>20/38 (52.6)</td>
<td>30.0, 40.0, —</td>
<td>6/4</td>
<td>14d (33.3)</td>
<td>16d (33.3)</td>
</tr>
<tr>
<td>Martin et al. (18)</td>
<td>China</td>
<td>2007-2008</td>
<td>NA</td>
<td>Primary</td>
<td>Primary ulcer</td>
<td>bpa, tpp47, polA</td>
<td>38/57 (66.7)</td>
<td>94.7, 100.0 —</td>
<td>36/4</td>
<td>14d (83.3)</td>
<td>NA</td>
</tr>
<tr>
<td>Pillay et al. (26)</td>
<td>South Africa</td>
<td>1996-2000</td>
<td>NA</td>
<td>Primary</td>
<td>Primary ulcer</td>
<td>polA</td>
<td>20/1954 (10.3)</td>
<td>80.9, 87.1, —</td>
<td>161/35</td>
<td>14d (27.3)</td>
<td>NA</td>
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<tr>
<td>Molepo et al. (23)</td>
<td>South Africa</td>
<td>1999-2000</td>
<td>NA</td>
<td>Primary</td>
<td>Primary ulcer</td>
<td>tpp47 or polA</td>
<td>28/50 (56.0)</td>
<td>46.4, 53.6, —</td>
<td>13/4</td>
<td>14a (53.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Florindo et al. (9)</td>
<td>Portugal</td>
<td>2004-2007</td>
<td>NA</td>
<td>Primary, secondary</td>
<td>Primary ulcer</td>
<td>tpp47, bmp, polA</td>
<td>86/NA (NA)</td>
<td>42/3</td>
<td>14a (50.0)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Castro et al. (3)</td>
<td>Portugal</td>
<td>2003-2005</td>
<td>NA</td>
<td>Primary, secondary, latent</td>
<td>Primary ulcer, secondary lesion, whole blood, serum, ear lobe scraping</td>
<td>polA</td>
<td>90/212 (42.5)</td>
<td>NA</td>
<td>20/5</td>
<td>14a (65.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Cole et al. (7)</td>
<td>UK</td>
<td>2006-2007</td>
<td>74.7</td>
<td>Primary, secondary</td>
<td>Genital ulcer, oral ulcer</td>
<td>polA</td>
<td>75/87 (86.2)</td>
<td>81.3, 85.3, —</td>
<td>58/6</td>
<td>14d (75.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Azzato et al. (1)</td>
<td>Australia</td>
<td>2004-2009</td>
<td>NA</td>
<td>NA</td>
<td>Oral ulcer, anorectal ulcer, lesion swabs, CSF, tissue biopsy specimen</td>
<td>polA</td>
<td>303/3652 (8.2)</td>
<td>57.1, NA —</td>
<td>90/10</td>
<td>14e (31.1)</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup>Among patients in Seattle with available demographic data.
<sup>b</sup>CNS, central nervous system.
<sup>c</sup>NA, not available.
<sup>d</sup>—, typing not done.
DISCUSSION

In this study, using the enhanced molecular typing method proposed by Marra and colleagues (17), we found that subtype 14f/f accounted for 50% of the fully typeable specimens in patients with syphilis in Taiwan, and the A2058G and A2059 point mutations, which are associated with azithromycin resistance, were not detected in this study.

Molecular typing can provide additional information in epidemiological investigations. Previous studies have described the geographic distribution of molecular subtypes of *T. pallidum* (Table 3). Subtype 14f was the most common subtype observed using the conventional typing method in the United States and China in studies reported in 2005 and 2009, respectively (18, 27, 30). In our present study, we found a predominance of subtype 14f/f. This result is different from the findings of the last two studies that also used the enhanced molecular typing method, both of which showed 14d/f as the most common subtype from 1999 to 2010 in the United States and China (Table 3) (17, 24). In this study, we found two new subtypes, 90/i and 10b/a, that had not been reported before. These findings suggest that currently circulating *T. pallidum* strains in Taiwan may be unrelated to those in the United States and China as determined by the enhanced molecular typing methodology.

Manifestations of syphilis may be associated with specific *T. pallidum* subtypes. In the study by Marra et al., subtype 14f/f was found to be associated with neurosyphilis (17). In this study, we found that subtype 14f/f was statistically significantly associated with primary syphilis. The cause of this association remains unclear. One possible explanation for this finding is that subtype 14f/f tends to produce chancres that are more manifest or last longer for patients or physicians to notice. It is also speculated that 14f/f subtypes are more environmentally resistant in swab specimens or during transportation. While the clinical implications remain unclear, more studies are warranted to confirm our preliminary findings.

Prior studies reported different prevalences of genotypic resistance of *T. pallidum* to macrolides, which were 2% in British Columbia during 2000 to 2003 (23), 88% in Dublin in 2002 (15), and 16 to 28.6% in Canada (18, 19), 37 to 68% in San Francisco (13, 21), and 100% in China (18). In this 2-year survey in Taiwan, no A2058G point mutation was found in the 211 clinical specimens, which was similar to the finding of the study by Van Damme and colleagues that demonstrated no evidence of azithromycin resistance of *T. pallidum* in Madagascar (31). The absence of a A2058G or A2059G point mutation in *T. pallidum* strains in this study may be partly explained by the findings of studies by Marra et al. and Mitchell et al. that macrolide resistance in multiple strains resulted from antibiotic pressure (16, 21). In our study, only one patient had a history of exposure to macrolide antibiotics prior to the diagnosis of syphilis. Patient-delivered partner treatment with macrolide antibiotics has not been implemented as a control strategy in preventing syphilis or *Chlamydia trachomatis* infection during and before our study period in Taiwan. Our finding suggests that azithromycin remains an appropriate alternative treatment for early syphilis in nonpregnant patients in Taiwan who are intolerant of penicillins. Nevertheless, caution should be taken and surveillance of drug-resistant *T. pallidum* is continued because of increased international travel and cross-strait travel between Taiwan and mainland China, where 100% of the *T. pallidum* strains were reportedly resistant to macrolides (18).

There are several limitations of our study, and interpretation of the results should be cautious. First, all specimens were obtained from patients in medical centers designated for HIV care, and therefore, the majority of the subjects were HIV-infected MSM. The results of molecular typing may not be representative of the *T. pallidum* strains that circulate in heterosexual or HIV-uninfected populations. Second, the number of clinical specimens that were fully typed using enhanced molecular typing methods remains small in our study compared to the two previously reported studies (Table 3). Continued surveillance among the participating hospitals is needed. Third, we used the VDRL and TPHA tests but not dark-field microscopy to confirm the diagnosis of syphilis. We might have missed some cases of primary syphilis. Last, this study was designed as a cross-sectional survey, which precluded us from following the patients longitudinally to investigate the changes of subtypes of *T. pallidum* in those patients with treatment failure.

In conclusion, we found that subtype 14f/f was the most common subtype of *T. pallidum* in Taiwan and that none of the strains harbored an A2058G or A2059G point mutation associated with macrolide resistance in this 2-year survey.

ACKNOWLEDGMENT

We have no conflicts of interest to declare.

REFERENCES

6. Reference deleted.