Prevalence of the 23S rRNA A2058G Point Mutation and Molecular Subtypes in *Treponema pallidum* in the United States, 2007 to 2009

The A2058G Prevalence Workgroup

**Background:** The 23S rRNA A2058G point mutation in *Treponema pallidum* is associated with macrolide antibiotic treatment failure. Its prevalence and potential association with a molecular subtype within the United States are unknown.

**Methods:** During 2007 to 2009, 11 clinics across the United States sent samples from genital ulcers to the Centers for Disease Control and Prevention. Molecular techniques were used to identify *T. pallidum* DNA sequences, the A2058G mutation, and subtype of *T. pallidum*. Accompanying epidemiologic information was abstracted from medical records.

**Results:** A total of 141 samples with *T. pallidum* were collected from individuals whose median age was 33 years (range, 13–68 years): 118 were male (69% reported as men having sex with men [MSM]). The A2058G mutation was carried in 75 samples (53%) with *T. pallidum*, with samples from MSM (versus women and other men) more likely carrying the A2058G mutation (65/82 samples versus 8/57 samples; prevalence ratio, 5.7; 95% confidence interval, 2.9–10.8). Of 98 strain-typed samples, 61 (62%) were the 14d9 subtype of *T. pallidum*, which was also associated with samples with *T. pallidum* from MSM (prevalence ratio, 3.5; 95% confidence interval, 1.9–6.5). However, among *T. pallidum* from MSM, the A2058G mutation was not associated with the 14d9 subtype.

**Conclusions:** The A2058G mutation and 14d9 subtype of *T. pallidum* were present throughout the United States. Both were more commonly found in *T. pallidum* from MSM compared with women or other men but were not associated with each other. Treating syphilis with azithromycin should be done cautiously and only when treatment with penicillin or doxycycline is not feasible.

Benzathine penicillin is the recommended treatment for syphilis. Alternative antimicrobials have also been used, including tetracycline, doxycycline, ceftriaxone, and various macrolides including erythromycin and azithromycin. Azithromycin is an appealing alternative because of the convenience of single-dose oral administration and its demonstrated efficacy in randomized, controlled trials. In studies conducted in Asia, Africa, Europe, and the United States, cure rates achieved with oral azithromycin were equivalent to rates achieved with benzathine penicillin. Other reports describe the efficacy of oral azithromycin in preventing incubating syphilis and in initially reducing cases in an outbreak of syphilis in Canada.

Despite its efficacy, case reports and small case series have reported syphilis treatment failures with azithromycin. One group has described a high and possibly increasing prevalence of a previously described A to G mutation at the position cognate to A2058 in the *Escherichia coli* 23S rRNA gene that was associated with a treatment failure and macrolide resistance in a 1968 clinical isolate of *Treponema pallidum*. This same A2058G mutation was associated with treatment failure in another patient. The A2058G mutation now seems widely dispersed, having been described in isolates of *T. pallidum* in the United States, Canada, Europe, and Asia.

Until recently, methods to evaluate *T. pallidum* strains and their associations with syphilis epidemiology were limited. In 1998, Pillay et al. described molecular techniques allowing the classification of *T. pallidum* into different subtypes. Investigators have used these molecular typing methods to describe variations in subtypes predominating in different geographic locations and patient populations. We have now applied these molecular typing methods to provide further insight into the epidemiology of *T. pallidum*, particularly *T. pallidum* with the A2058G mutation, across 11 cities in the United States.

**Materials and Methods**

A convenience sample of 10 clinics for the treatment of sexually transmitted diseases and 1 human immunodeficiency virus (HIV) care clinic (in Atlanta) meeting a threshold of syphilis morbidity (a 3-year average of ≥14 cases of primary syphilis per year during 2003–2005) were asked to submit samples to the Centers for Disease Control and Prevention (CDC) during 2007.
to 2009. These sites were located in geographic regions across the United States except for the Northeast, where no sites participated. Participating clinics were located in San Diego, CA; San Francisco, CA; Seattle, WA; Phoenix, AZ; Albuquerque, NM; Chicago, IL; Detroit, MI; Dallas, TX, Birmingham, AL; Atlanta, GA; and Baltimore, MD. Participating sites also provided data describing the patients from whom these samples were obtained, including demographic information (sex, age, and race/ethnicity), sex of sex partner, and HIV status. Each sample came from a unique individual.

Sample collection methods varied. In most participating sites, samples were taken from specimens of genital ulcers submitted for dark field microscopy after their intended clinical use and put into Genelock® medium (Sierra Molecular Corporation, Sonora, CA); in Albuquerque, NM, samples were aliquots taken from herpes culture viral transport media. All samples were shipped to the CDC laboratories in either Genelock® (Sierra Molecular Corporation) or viral transport media. DNA was extracted from these samples and amplified by real-time polymerase chain reaction (PCR) for evidence of T. pallidum as described previously.16 Using established methods,14,17,18 samples positive for T. pallidum underwent further analysis to determine if the sample carried the 23S rRNA A2058G point mutation; samples positive for T. pallidum also underwent strain typing.

Molecular strain typing of T. pallidum was performed using a modification of previously described typing methods.14,18 Briefly, real-time PCR amplification products of the tprE [tp0313], G [tp0317], and J [tp0621] genes were digested with MseI restriction enzyme and analyzed. The number of 60-base-pair tandem repeats within the arp gene was determined using real-time PCR amplification with primers N1 (5′ ATCTTTGCCGTCCC[CI] 1.3; 95% confidence interval [CI], 1.3–7.5; P = 0.002). Although overall median age was 33 years (range 13–68), the men tended to be slightly older than the women (median ages of 34 and 29 years, respectively) and tended to have a more diversified racial/ethnic distribution relative to the women (men: 36% non-Hispanic white, 31% non-Hispanic black, 22% Hispanic, and 11% other; women: 10% non-Hispanic white, 81% non-Hispanic black, 0% Hispanic, and 9% other).

Of the 118 men, 82 (69%) reported sex with men (MSM) (including 3 who reported sex with both men and women), and 36 (31%) reported sex exclusively with women (MSW). Among men, 65 (79%) of 82 MSM had T. pallidum carrying the tpa279 gene. Incorporation of the homonucleotide tandem repeat analysis yields added discrimination of T. pallidum subtypes.

Univariate analysis included Yates χ² and Fisher exact tests (where appropriate) using EpInfo version 7 (Atlanta, GA).

The activities of this project were determined not to be research but considered part of routine disease surveillance. This project was thus exempt from institutional review board review.

### RESULTS

#### T. pallidum

Participating sites submitted a total of 651 samples to the CDC laboratory. The number of samples that tested PCR positive for T. pallidum ranged by site from 1 to 36 (Table 1). A total of 141 samples (21.6%) tested PCR positive for T. pallidum; 75 (53.2%) carried the 23S rRNA A2058G point mutation (Table 1). Samples carrying the A2058G mutation came from 9 (81.8%) of 11 participating sites. Two sites, both in the Midwest, did not have samples carrying the A2058G mutation; however, only 14 samples were collected from these sites.

Complete demographic data were available for 139 (99%) of 141 participants (118 men and 21 women) having samples positive for T. pallidum; race/ethnicity data were available for all men (100%) and 95% (20/21) for women. Of the 118 men, 69 (58%) had T. pallidum with the A2058G mutation, whereas only 4 (19%) of 21 women had T. pallidum with the A2058G mutation (prevalence ratio [PR], 3.1; 95% confidence interval [CI], 1.3–7.5; P = 0.002). Although overall median age was 33 years (range 13–68), the men tended to be slightly older than the women (median ages of 34 and 29 years, respectively).

#### TABLE 1. Samples Carrying the 23S rRNA A2058G Point Mutation, by Region of the United States and Site

<table>
<thead>
<tr>
<th>Region and Site</th>
<th>Samples Submitted (n = 651)</th>
<th>Samples PCR-Positive for T. Pallidum (Percentage of All Sites) (n = 141)</th>
<th>Samples With T. Pallidum Carrying the A2058G Mutation (Percentage of All Sites) (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West</td>
<td>344</td>
<td>82 (58%)</td>
<td>63 (84%)</td>
</tr>
<tr>
<td>Albuquerque, NM</td>
<td>233</td>
<td>10 (7%)</td>
<td>8 (11%)</td>
</tr>
<tr>
<td>Phoenix, AZ</td>
<td>22</td>
<td>9 (6%)</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>San Diego, CA</td>
<td>36</td>
<td>17 (12%)</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>San Francisco, CA*</td>
<td>37</td>
<td>36 (26%)</td>
<td>28 (37%)</td>
</tr>
<tr>
<td>Seattle, WA</td>
<td>16</td>
<td>10 (7%)</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>Midwest</td>
<td>39</td>
<td>14 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chicago, IL</td>
<td>5</td>
<td>1 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Detroit, MI</td>
<td>34</td>
<td>13 (9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>South</td>
<td>268</td>
<td>45 (32%)</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>Dallas, TX</td>
<td>13</td>
<td>9 (6%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Birmingham, AL</td>
<td>35</td>
<td>21 (15%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Atlanta, GA</td>
<td>10</td>
<td>4 (3%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Baltimore, MD</td>
<td>210</td>
<td>11 (8%)</td>
<td>5 (7%)</td>
</tr>
</tbody>
</table>

*Samples from San Francisco came only from dark field–positive specimens.
the A2058G mutation, whereas only 4 (11%) of 36 MSW had *T. pallidum* carrying the mutation (PR, 7.1; 95% CI, 2.8–18.1; \( P < 0.001 \)). Compared with MSW and women in aggregate, MSM were more likely to have *T. pallidum* with the A2058G mutation (PR, 5.7; 95% CI, 2.9–10.8).

HIV status was available for 71 MSM (of whom 26 [37%] were HIV-positive), 35 MSW (of whom 1 [3%] was HIV-positive), and 19 women (of whom 1 [5%] was HIV-positive). HIV-positive MSM were no more likely to carry *T. pallidum* with the A2058G mutation than HIV-negative MSM (PR, 0.8; 95% CI, 0.6–1.1; \( P = 0.08 \)).

Most samples from MSM came from the West (Table 2), whereas most samples from MSW came from the South. One consideration is whether the A2058G mutation might be associated more with geographic region or race than with sex of partner. However, focusing upon the South alone (from where most of the samples from MSW came), 7 (77.8%) of 9 MSM had *T. pallidum* with the A2058G mutation, whereas only 2 (9.5%) of 21 MSW had *T. pallidum* with the mutation (PR, 8.2; 95% CI, 2.1–31.9; \( P < 0.001 \)). Considering only black men in the South, 3 of 5 MSM (60.0%) had *T. pallidum* with the A2058G mutation, whereas only 2 of 19 MSW (10.5%) had *T. pallidum* with the mutation (PR, 5.7; 95% CI, 1.3–25.4; \( P = 0.04 \)).

### Molecular Subtyping

Ninety-nine of one hundred and forty-one samples (70%) could be subtyped; 12 different subtypes of *T. pallidum* were identified (Fig. 1). The common *T. pallidum* type 1A4d was actually comprised of 3 subtypes (14d9, 14d11, 14d12). MSM with *T. pallidum* type 1A4d had the 14d9 subtype only, whereas MSW and women also had *T. pallidum* of the 14d11 and 14d12 subtypes, respectively. Subtype 14d9 accounted for most (62%) of the 99 subtyped samples. 53 of 64 (83%) MSM in this project had the 14d9 subtype. Compared with MSW and women, MSM more frequently had the 14d9 subtype (PR, 3.5; 95% CI, 1.9–6.5; \( P < 0.001 \)).

Although the 14d9 subtype predominated among MSM, different and wider distributions of subtypes of *T. pallidum* were observed among MSW and women. Eight subtypes of *T. pallidum* were observed among MSW, 5 (62.5%) of which were not observed among MSM. Six subtypes of *T. pallidum* were observed among women, with no subtype predominating.

Of the 99 subtyped samples, 58 (59%) carried the A2058G mutation; of these 58 samples, 48 (83%) were the 14d9 subtype, 6 (10%) were the 14d9 subtype, 2 (3%) were the 14p9 subtype, and 2 (3%) were the 15d12 subtype. However, the A2058G mutation was commonly found among both non-14d9 and 14d9 subtypes among MSM (Fig. 2). No association between the 14d9 subtype and the A2058G mutation was observed among MSM.

### DISCUSSION

Despite its apparent efficacy as an alternative treatment for syphilis, 2,3 treatment failures with azithromycin have now been reported. 5–8 The 23S rRNA A2058G point mutation associated with resistance to azithromycin has now been reported on at least 3 continents. 6,9,13 This report demonstrates that both this mutation and the *T. pallidum* subtypes in which the mutation is commonly found are present across multiple locations in the United States. Both the A2058G mutation and the 14d9 *T. pallidum* subtype were found more frequently among MSM compared with MSW and women. No association between the A2058G mutation and 14d9 subtype was observed among MSM.

Although our specimens were the result of convenience samples, the racial or ethnic distribution of MSM and MSW with primary and secondary (P&S) syphilis in this report resembles

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**TABLE 2.** Regional Distribution of MSM* and MSW,* by Race/Ethnicity and 23S rRNA A2058G Point Mutation Status

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>MSM A2058G (+)</th>
<th>MSM A2058G (−)</th>
<th>MSW A2058G (+)</th>
<th>MSW A2058G (−)</th>
<th>Total (n = 82)</th>
<th>West A2058G (+)</th>
<th>West A2058G (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>White (n = 5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Black (n = 5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hispanic (n = 2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Other (n = 5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*MSM denotes men having sex with men; MSW denotes men having sex with women only.*

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**Figure 1.** *T. pallidum* subtypes, by sex and sexual behavior. Subtype 14d9 predominates among MSM, whereas a wider distribution of subtypes is present among MSW and women. *MSM denotes men having sex with men; MSW denotes men having sex with women only.*
The median &T. pallidum (specifically, subtype 14)
Mycoplasma pneumoniae in MSM and the A2058G mutation
However, the observed predominance
Supporting this possibility, a previ-
has been reported in Maricopa County, AZ, and in
T. pallidum T. pallidum subtypes observed in this project supports this
might
Current guidelines
T. pallidum T. pallidum of type 14
Because the molecu-
type 14 Chlamydia trachu-
and other types existed in the United
in other areas of the United States differs from previously
Similarly, the lower
The distribution of
from MSM.
797
Given the global dissemination of the A2058G muta-
Sutton et al.
The
in Australia.
are also associated with
possibly because of the anatomic location of the pain-
MSW denotes men having sex with women only.
distributions reported in the United States—specifically, that in
2009, MSM accounted for 62% of reported cases of P&S syphilis
and that, among MSW and women, blacks accounted for more
cases than all other races or ethnicities combined.19 The median
age of MSM in this report also reflect the great numbers of
young men reported with P&S syphilis.19 Similarly, the lower
numbers of samples from women in general and the relatively
greater proportion of samples from black MSW from the South
are consistent with current US syphilis epidemiology.20 The
samples in this report predominantly came from specimens for
dark field microscopy, mostly primary syphilitic chancres. Only a
small proportion of infectious syphilis reported among women
is primary syphilis (compared with syphilis reported among MSW or
MSM)19 possibly because of the anatomic location of the pain-
less primary chancre. This observation might, in part, explain
the lower number of samples from women.

Despite regional differences among persons infected with
syphilis by race, ethnicity, and sexual behavior, a clear associa-
tion between T. pallidum in MSM and the A2058G mutation
was observed (Table 2). This association remains significant
even when limiting consideration to samples from black men
in the South, suggesting that this association is independent of
both race/ethnicity and geographic region. These observations
agree with previous reports describing the frequent isolation
of the A2058G mutation among T. pallidum from MSM.7,21
A clear explanation for this association with MSM remains
classic. One possible reason might be greater use of azithromy-
cin among MSM (relative to MSW or women),7,9 for example,
for prophylaxis against Mycobacterium avium complex in
HIV-positive individuals.22 Supporting this possibility, a previ-
ous report demonstrates that resistance to azithromycin can arise
through selective pressure.23 However, T. pallidum from HIV-
positive MSM were not more likely to carry the A2058G muta-
tion than T. pallidum from HIV-negative MSW. Alternatively,
the association might reflect the transmission of T. pallidum
strains within sexual networks largely limited to MSM—T. pallidum
carrying the A2058G mutation might be highly prevalent among
networks of sexual partners of MSM in different parts of the
country. Direct transmission of T. pallidum already carrying
the A2058G mutation has been reported.7 The distribution of
T. pallidum subtypes observed in this project supports this
possibility (Fig. 1), and the use of alternative typing methods
for T. pallidum, as recently described by Marra et al.,24 might
yield insights into this possibility.

How frequently the A2058G mutation is associated with
macrolide treatment failure remains unknown. No prospective
data exist describing patients who had T. pallidum carrying the
A2058G mutation and were subsequently treated with macro-
liges. The A2058G mutation has been associated with macrolide
treatment failure or shown to confer resistance in vitro among other
pathogens, including Neisseria gonorrhoeae, Chlamydia trach-
matis, and Mycoplasma pneumoniae.25–27 Current guidelines
for the treatment of syphilis emphasize that azithromycin should
only be used if penicillin- or doxycycline-based therapies are not
feasible.1 Given the global dissemination of the A2058G muta-
tion and the data presented here, concern about any routine use
of azithromycin to treat syphilis is warranted and caution in
using azithromycin (or other macrolide antibiotics) to treat syph-
ilis seems appropriate, especially among MSM.

T. pallidum type 14 (specifically, subtype 14e9) repre-
sented the majority of samples among MSM in this report
(Table 2), consistent with recent reports from Scotland, Seattle,
and San Francisco.18,24,28 However, the observed predominance
of 14e in other areas of the United States differs from previously
observed subtypes. Pillay et al.14 noted in 1998 that although
T. pallidum of type 14e and other types existed in the United
States, no single type was predominant. A predominance of
type 14f has been reported in Maricopa County, AZ, and in
counties of North and South Carolina, despite racial or ethnic
gender differences.15,29 Outside of the United States,
investigators have reported the predominance of types 14e in
Lisbon, Portugal, and of 14e in Australia.30,31

These differences might reflect the changing epidemiolo-
gy of syphilis, with a greater proportion of cases among MSM
comparing with cases reported in the 1990s.18,24,28 Sutton et al.29
collected samples mostly from Hispanic and white MSW during
1997 to 1998, whereas Pope et al.15 collected samples mostly
from black patients during 1999 to 2003. In the present report,
samples came primarily from white and Hispanic MSM and
black MSW during 2007 to 2009. Whether this changing epide-
miology can explain these differences and their significance
will require further investigation.

These data have limitations. Local epidemiology might
influence some of the data presented here and preclude infer-
rance of the prevalence of both the A2058G mutation and subtype
of T. pallidum beyond the sites described in this report. Of the
34 samples from MSW, 27 (79%) came from black MSW. These
data might not adequately reflect the dispersion of the A2058G
mutation and/or subtypes of T. pallidum in MSW of other racial
and ethnic groups. Nonetheless, most P&S syphilis among
MSW in the United States occurs among black MSW,32 and pre-
vious reports support the association of MSM with both the
A2058G mutation and the 14e9 subtype reported here.

Other mutations in T. pallidum are also associated with
treatment failure with macrolides. A second mutation associated
with macrolide treatment failure has been identified recently
(a 23S rRNA A2059G point mutation).6 Because the molecu-
lar methods used in this project were not designed to detect an
A2059G mutation, some samples identified as A2058G(1) might
harbor the A2059G mutation and hence be associated
with macrolide treatment failure as well. Understanding the dis-
tribution of this novel mutation will require further investigation.

However, the presence of a second mutation in T. pallidum as-
sociated with macrolide treatment failure reinforces the recom-
mendation for caution in using azithromycin or other macrolide
antibiotics to treat syphilis.
In summary, we report the wide dissemination of the 23S rRNA A2058G point mutation associated with azithromycin resistance across several states in the United States. *Treponema pallidum* from MSM are more likely to carry this mutation than *T. pallidum* from MSW or women, and providers should be aware that resistance to azithromycin associated with the A2058G mutation can also occur in these populations. Among MSM, the 14d9 subtype is predominant but not associated with the A2058G mutation; these observations might reflect differences in sexual behaviors (or networks of sexual partners). The clinical significance of both observations requires further investigation.

REFERENCES


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