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 14*d*9 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 14*d*9 subtype.

Conclusions: The A2058G mutation and 14*d*9 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.[1] 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.[2,3] Other reports describe the efficacy of oral azithromycin in preventing incubating syphilis and in initially reducing cases in an outbreak of syphilis in Canada.[4,5]

Despite its efficacy, case reports and small case series have reported syphilis treatment failures with azithromycin.[6–8] One group has described a high and possibly increasing prevalence[9] 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*.[10,11] This same A2058G mutation was associated with treatment failure in another patient.[9] The A2058G mutation now seems widely dispersed, having been described in isolates of *T. pallidum* in the United States, Canada, Europe, and Asia.[6,9,12,13]

Until recently, methods to evaluate *T. pallidum* strains and their associations with syphilis epidemiology were limited. In 1998, Pillay et al.[14] 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.[15] 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

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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 *Mse*I 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 GTGTGC3′) and N2 (5′CCGAGTGGGATGGCTGCTTC3′). In addition, the homonucleotide G tandem repeat within *tp0279* was sequenced and used to further discriminate among strains.[18] Subtypes were characterized by combining the results for all 3 methods. For example, using this strain typing method, the Nichols strain of *T. pallidum* is identified as subtype 14*a*10, where "14" is the number of 60-base-pair repeats within the *arp* gene, "*a*" is the restriction fragment length polymorphism pattern of the PCR amplicon of the *tprE*, *G*, and *J* genes, and "10" is the number of tandem repeat G residues within the *tp0279* gene. Incorporation of the homonucleotide tandem repeat analysis yields added discrimination of *T. pallidum* subtypes.

Univariate analysis included Yates χ^2 and Fisher exact tests (where appropriate) using EpiInfo 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

A2058G Mutation

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) 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

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

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

*Samples from San Francisco came only from dark field–positive specimens.

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

	Midwest A2058G(+)	Midwest A2058G(−)	South A2058G(+)	South A2058G(−)	West A2058G(+)	West A2058G(−)
MSM						
White (n = 38)	0	0	2	0	28	8
Black (n = 8)	0	0	3	2	2	1
Hispanic (n = 24)	0	0	2	0	18	4
Other (n = 12)	0	0	0	0	10	2
Total (n = 82)	0	0	7	2	58	15
MSW						
White (n = 5)	0	0	0	1	2	2
Black (n = 28)	0	9	2	17	0	0
Hispanic (n = 2)	0	0	0	0	0	2
Other (n =1)	0	0	0	1	0	0
Total (n = 36)	0	9	2	19	2	4

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

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 14*d* was actually comprised of 3 subtypes (14*d*9, 14*d*11, 14*d*12). MSM with *T. pallidum* type 14*d* had the 14*d*9 subtype only, whereas MSW and women also had *T. pallidum* of the 14*d*11 and 14*d*12 subtypes, respectively. Subtype 14*d*9 accounted for most (62%) of the 99 subtyped samples. 53 of 64 (83%) MSM in this project had the 14*d*9 subtype. Compared with MSW and women, MSM more frequently had the 14*d*9 subtype (PR, 3.5; 95% CI, 1.9–6.5; $P < 0.001$).

Although the 14*d*9 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 14*d*9 subtype, 6 (10%) were the 14*e*9 subtype, 2 (3%) were the 14*p*9 subtype, and 2 (3%) were the 15*d*12 subtype. However, the A2058G mutation was commonly found among both non-14*d*9 and 14*d*9 subtypes among MSM (Fig. 2). No association between the 14*d*9 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 been reported.[6–8] 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 14*d*9 *T. pallidum* subtype were found more frequently among MSM compared with MSW and women. No association between the A2058G mutation and 14*d*9 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

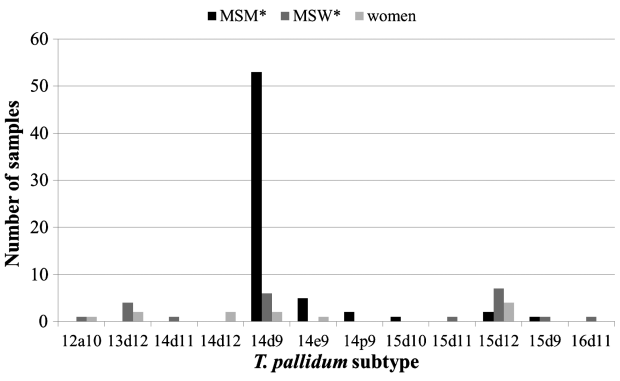

Figure 1. *T. pallidum* subtypes, by sex and sexual behavior. Subtype 14*d*9 predominates among MSM, whereas a wider distribution of subtypes is present among MSW and women. *MSM denotes men having sex with men; MSW denotes men having sex with women only.

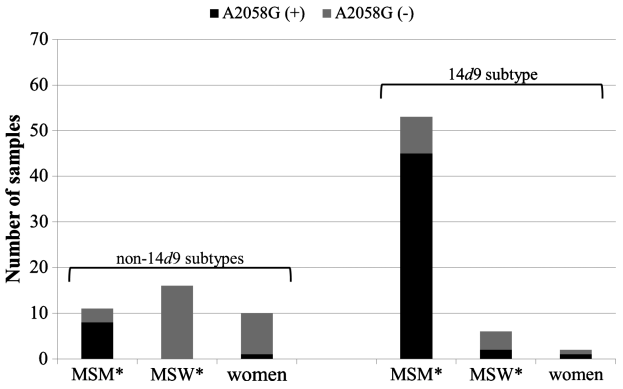

Figure 2. Presence (+) or absence (−) of A2058G mutation among non-14*d*9 and 14*d*9 subtypes of *T. pallidum*, by sex and sexual behavior. The A2058G mutation is present among most samples of *T. pallidum* from MSM, regardless of subtype. *MSM denotes men having sex with men; MSW denotes men having 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 painless 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 association 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 elusive. One possible reason might be greater use of azithromycin 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 previous 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 mutation than *T. pallidum* from HIV-negative MSM. 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 macrolides. The A2058G mutation has been associated with macrolide treatment failure or shown to confer resistance in vitro among other pathogens, including *Neiserria gonorrhoeae*, *Chlamydia trachomatis*, 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 mutation 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 syphilis seems appropriate, especially among MSM.

T. pallidum type 14*d* (specifically, subtype 14*d*9) represented 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 14*d* 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 14*d* and other types existed in the United States, no single type was predominant. A predominance of type 14*f* has been reported in Maricopa County, AZ, and in counties of North and South Carolina, despite racial or ethnic and geographic differences.[15,29] Outside of the United States, investigators have reported the predominance of types 14*a* in Lisbon, Portugal, and of 14*e* in Australia.[30,31]

These differences might reflect the changing epidemiology of syphilis, with a greater proportion of cases among MSM compared with cases reported in the 1990s.[32,33] 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 epidemiology 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 precludes inferring 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,[19] and previous reports support the association of MSM with both the A2058G mutation and the 14*d*9 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 molecular methods used in this project were not designed to detect an A2059G mutation, some samples identified as A2058G(−) might harbor the A2059G mutation and hence be associated with macrolide treatment failure as well. Understanding the distribution of this novel mutation will require further investigation. However, the presence of a second mutation in *T. pallidum* associated with macrolide treatment failure reinforces the recommendation 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. *T. pallidum* from MSM are more likely to carry this mutation than *T. pallidum* from MSW or women, validating concerns about using azithromycin to treat syphilis in MSM. The A2058G mutation is also found in *T. pallidum* from women and MSW, and providers should be aware that resistance to azithromycin associated with the A2058G mutation can also occur in these populations. Among MSM, the 14*d*9 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.

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