Molecular Epidemiology of Syphilis—San Francisco, 2004–2007

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Abstract: We describe the molecular epidemiology of syphilis in San Francisco (SF) using *Treponema pallidum* specimens obtained from patients examined at the SF municipal sexually transmitted diseases clinic during 2004–2007. Of 69 specimens, 52 (75%) were subtype 14*d*9. Single subtype predominance might reflect a closely linked sexual network in SF.

Syphilis is a sexually transmitted disease (STD) caused by the bacterium *Treponema pallidum* subspecies *pallidum (T. pallidum*). The incidence of primary and secondary syphilis (P&S) in the United States increased by 114% from 2000 (2.1 cases/100,000 population) to 2008 (4.5 cases/100,000 population).[1] In San Francisco (SF), the incidence of P&S syphilis increased >600%,[2,3] from 3.8 cases/100,000 population in 1999 to 26.0 cases/100,000 population in 2007, largely reflecting an epidemic among men who have sex with men (MSM). During that time, azithromycin resistance in *T. pallidum* emerged in SF and other areas,[4] with 77.3% of *T. pallidum* specimens obtained from SF syphilis patients in 2006 harboring the gene associated with azithromycin resistance.[5]

Molecular epidemiology has proven useful in preventing and controlling certain infectious diseases. Genotyping of *Mycobacterium tuberculosis*, for example, has identified patients not captured by routine public health investigations,[6] and pulsefield gel electrophoresis-based molecular typing has enhanced infectious foodborne illness surveillance.[7,8] By contrast, molecular epidemiologic methods have yet to gain routine use in STD prevention and control, although those methods have been used in studies of gonorrhea.[9,10]

Since 1998, molecular characterization of *T. pallidum* has entailed analysis of 2 genes to type the organism and, more recently, analysis of a third gene to determine its subtype.[11,12] This typing system has been applied to specimens obtained from skin and mucous membrane lesions,[13–17] blood,[15,17,18] and cerebrospinal fluid[18,19] among patients from North Carolina and South Carolina,[14] Arizona,[15] and Seattle[18] in the United States and South Africa,[13,19] Portugal,[20] Scotland,[16] and China.[17]

In this study, we described the distribution of *T. pallidum* subtypes causing incident P&S syphilis in SF and determined whether *T. pallidum* subtype correlated with characteristics of syphilis patients or their infections, including molecular markers associated with resistance to azithromycin.

T. pallidum specimens were obtained by swabbing darkfield-positive primary or moist secondary syphilis lesions of patients examined at the San Francisco City Clinic (SFCC), SF municipal STD clinic, during November 19, 2004–November 21, 2007. Patient characteristics were captured by using public health records.

To detect the presence of *T. pallidum* in swab specimens, we used a real-time diagnostic polymerase chain reaction (PCR) assay that amplifies a segment of the *polA* gene of *T. pallidum*.[21] To subsequently type specimens, we used PCR to determine the number of 60-bp tandem repeats within the *arp* gene by PCR, using primers 1A and 2A, and PCR-restriction fragment length polymorphism (RFLP) analysis of the *tpr E*, *G*, and *J* genes, as described previously,[11,13] except that all PCR amplicons in this study were analyzed on an Agilent 2,100 Bioanalyzer (Agilent Technologies, San Diego, CA).[11,13] To type specimens positive by the *polA* assay but negative by the original *arp* assay,[11] we used an alternate primer set N1 (5′ ATCTTTGCCGTCCCGTGTGC3′) and N2 (5′CCGAGTGGGATGGCTGCTTC3′), with touchdown PCR conditions. An initial cycle of 94°C for 2 minutes was performed, followed by stage one of the touchdown method, which consisted of 13 cycles of 94°C for 30 sec; 74°C for 45 seconds decreasing at 1°C per cycle; and 72°C for 1 minute 30 seconds. Stage 2 consisted of 25 cycles of 94°C for 30 seconds; 64°C for 45 seconds; and 72°C for 1 minute 30 seconds. Final extension was achieved at 72°C for 10 minutes. To subtype specimens,

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TABLE 1. Characteristics of Syphilis Patients and Treponema pallidum Azithromycin Resistance Status by T. pallidum Subtype (14d9 vs. Non-14d9)

Characteristic	Subtype 14*d*9 (n = 52)	Non-14*d*9 Subtypes (n = 17)	*P*∗
Age (yr), median (range)	41 (23–64)	43 (32–51)	0.57
Sex, number (%)			
Male	51 (98.1)	17 (100)	1.00
Male-to-female transgender	1 (1.9)	0 (0)	
Gender of sex partners,† number (%)			
Male only	42 (80.8)	16 (94.1)	0.18
Male and female	1 (2.3)	0 (0)	
Female only	1 (2.3)	1 (5.9)	
Unknown	8 (15.4)	0 (0)	
Race or ethnicity, number (%)			
White	29 (55.8)	13 (76.5)	0.40
Hispanic	16 (30.8)	2 (11.8)	
Black	4 (7.7)	1 (5.9)	
Asian	3 (5.9)	1 (5.9)	
Homeless, number (%)	0 (0)	1 (6.3)	0.25
HIV status at time of diagnosis			
HIV uninfected	29 (55.8)	9 (52.9)	0.84
HIV infected	22 (42.3)	8 (47.1)	
Missing	1 (1.9)	0 (0)	
Antibiotic use,‡ number (%)			
No	37 (71.2)	12 (70.6)	0.12
Yes	4 (7.7)	4 (23.5)	
Missing	11 (21.2)	1 (5.9)	
No. male sex partners,† median (range)	5.5 (0–75)	4 (0–30)	0.31
No. female sex partners,† median (range)	0 (0–2)	0 (0–2)	0.88
Sex work‡, number (%)			
No	44 (84.6)	16 (94.1)	0.75
Yes	1 (1.9)	0 (0)	
Missing	7 (13.5)	1 (5.9)	
Contact with sex worker,‡ number (%)			
No	43 (82.7)	16 (94.1)	0.81
Yes	2 (3.8)	0 (0)	
Missing	7 (13.5)	1 (5.9)	
Met sex partner(s) at the following venues,‡ number (%)			
Internet	19 (36.5)	9 (52.9)	0.27
Bar or club	7 (13.5)	1 (5.9)	0.67
Bath house or sex club	5 (9.6)	0 (0)	0.57
Bookstore	5 (9.6)	0 (0)	0.32
Methamphetamine use,‡ number (%)			
No	9 (17.3)	3 (17.6)	0.93
Yes	12 (23.1)	5 (29.4)	
Missing	31 (59.6)	9 (50.0)	
Travel outside continental United States,§ number (%)			
No	48 (92.3)	16 (94.1)	1.00
Yes	4 (7.7)	1 (5.9)	
Year of diagnosis, number (%)			
2004	8 (15.4)	0 (0)	0.14
2005	13 (25.0)	5 (29.4)	
2006	15 (28.8)	9 (52.9)	
2007	16 (30.8)	3 (17.6)	
Azithromycin resistance status			
Resistant	35 (67.3)	7 (41.2)	0.17¶
Sensitive	11 (21.2)	6 (35.3)	
DNA cannot be amplified	0 (0)	3 (17.6)	
Assay for resistance status not performed	6 (11.5)	1 (5.9)	
HIV: human immunodeficiency virus			

∗*t* and Fisher exact tests used for analyses of continuous and categorical variables, respectively.
†Within 3 and 6 mo for primary and secondary syphilis patients, respectively.
‡Within 1 yr before diagnosis.
§Within 60 d before diagnosis.
¶Comparing the proportions of resistant and sensitive specimens by subtype; does not include specimens for which DNA cannot be amplified or for which the assay was not performed.

we used PCR to determine the number of repeats in a homonucleotide G tandem repeat region within the *rpsA* gene (*tp0279*).[12] The 3-component subtype is represented by the number of *arp* repeats (e.g., "14" in 14*d*9, with possibilities ranging from 2–22), the *tpr E, G,* and *J* gene pattern (e.g., "*d*" in 14*d*9, with possibilities ranging from *a–p*), and the number of *rpsA* gene G repeats (e.g., "9" in 14*d*9, with possibilities ranging from 8–11).[11–13,16,18]

Separate swab specimens collected from each patient were analyzed for the A2058G mutation in the 23S ribosomal RNA gene associated with azithromycin resistance by using PCR amplification followed by restriction digestion.[4]

Patient characteristics and the molecular marker associated with azithromycin resistance in their specimens were compared between 14*d*9 and non-14*d*9 infections by using *t* and Fisher exact tests for continuous and categorical variables, respectively. All analyses were performed by using Stata 9.0 software (Stata Corporation, College Station, TX). This study was classified as a nonresearch public health activity by the San Francisco Department of Public Health and the Centers for Disease Control and Prevention.

Specimens were obtained from 74 patients, which include 70 (45%) of 155 patients with primary syphilis and 4 (2%) of 203 patients with secondary syphilis examined at SFCC during the 3-year study period. Of those, *T. pallidum* from 69 (93%) of 74 specimens, which includes 66 (94%) of 70 specimens from primary syphilis patients and 3 (75%) of 4 specimens from secondary syphilis patients could be fully subtyped. One specimen from a secondary syphilis patient could be partially subtyped. In one specimen from a primary syphilis patient, *T. pallidum* DNA could be detected but not typed or subtyped. *T. pallidum* DNA was not detected in 3 specimens from patients diagnosed with primary syphilis based on darkfield microscopy.

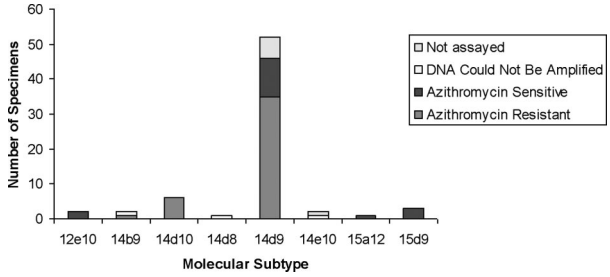

Figure 1. Molecular subtype and azithromycin resistance status of fully subtyped *Treponema pallidum* specimens (N = 69) obtained from primary and secondary syphilis patients examined at San Francisco City Clinic, 2004–2007.

T. pallidum subtype 14*d*9 was detected in 52 of 69 fully subtyped specimens (75%; 95% confidence interval [CI], 64%–84%) and subtype 14*d*10 in 6 of 69 (9%, 95% CI, 4%–18%) of fully subtyped specimens (Table 1). Subtype 15*d*9 accounted for 3 specimens; subtypes 12*e*10, 14*b*9, and 14*e*10 each accounted for two specimens; and subtypes 14*d*8 and 15*a*12 each accounted for one specimen. The partially subtyped specimen was classified as *d*10. Type 14*d* accounted for 59 (85.5%) of 69 specimens.

Of 62 fully subtyped specimens tested for the mutation associated with azithromycin resistance, 42 (68%) from 3 different subtypes were positive and 17 (27%) were negative; *T. pallidum* DNA was not detected in 3 swabs (5%) (Fig. 1). Selected patient characteristics and presence of the mutation conferring azithromycin resistance did not significantly differ between those with 14*d*9 infections, compared with those with non-14*d*9 infections (Table 1).

This study demonstrated that a high percentage of incident P&S syphilis cases examined at SFCC—76%—resulted from infection with subtype 14*d*9 strains of *T. pallidum*. The percentage of infections resulting from a single type (14*d*) in SF is slightly greater than that reported in Scotland[16] and China[17] and greater than that reported in other studies (Table 2).[13–15,18–20] The high incidence of subtype 14*d*9 infections in SF might have resulted from introduction of that subtype either before or at the beginning of the epidemic in SF. As suggested by a recent study of gonorrhea in London that reported fewer strains of *Neisseria gonorrhoeae* among MSM compared with heterosexuals, sexual networks of MSM can be more closely linked, allowing for acquisition of fewer strains that account for more cases.[22] Both the Scotland and China *T. pallidum* typing studies, which also reported high proportions of cases with a single type, included mostly men.[16,17]

We were unable to demonstrate statistically significant associations between *T. pallidum* subtypes and patient characteristics or the molecular marker associated with azithromycin resistance. That lack of associations might reflect limited statistical power, given that only 69 patients had fully subtypeable infections. Other studies have reported associations between *T. pallidum* type and patient characteristics. A study in Arizona reported an association between type 14*f* and white race.[15] In Shanghai, China, 5 types caused the 38 infections studied, all of which carried the mutation associated with azithromycin resistance.[17] Finally, a study in Seattle, WA, linked strain type to development of neurosyphilis.[18]

This study is subject to at least two additional limitations. First, the results might not be generalizable to SF as a whole because primary and secondary syphilis patients whose *T. pallidum* infections were subtyped represented only a minority of all P&S syphilis patients in SF during the study period. Second, we were unable to identify pairs of mutual sexual contacts with subtyped *T. pallidum* infections, which might have limited our ability to detect associations between subtypes and patient characteristics. This precluded our ability to assess the stability, during person-to-person transmission, of the genetic loci used in the subtyping system. However, long-term stability of the *arp* and *tpr E*, *G*, and *J* genes in rabbit-passaged and tissue-cultured *T. pallidum* specimens has previously been demonstrated.[11]

Molecular epidemiologic studies of syphilis have the potential, ultimately, to enhance clinical care and/or prevention and control efforts by contributing to a better understanding of *T. pallidum* acquisition and transmission. However, the difficulty of identifying sexual contacts and/or obtaining specimens for diagnostic PCR testing and typing purposes remains a challenge. Syphilis, unlike other sexually transmitted infections, has a long incubation period, early clinical manifestations that may be inapparent, and long periods of latency. All these factors contribute to difficulties in performing epidemiologic studies. Despite these challenges, the *T. pallidum* typing system

TABLE 2. Previous Studies Showing Distribution of Predominant T. pallidum Strain Type by Geographic Location

Author	Area(s) Studied	Stage of Disease	No. Specimens Typed	Most Common Type (% of All Specimens That Were of That Type)
Sutton et al[15]	Maricopa County, AZ	Primary or secondary syphilis	45	14*f* (53%)
Pillay et al[13]	Carletonville, Welkom, Johannesburg, Cape Town, and Durban, South Africa	Primary syphilis	161	14*d* (27%)
Pope et al[14]	Four counties in North and South Carolina	Primary or secondary syphilis	23	14*f* (52%)
Molepo et al[19]	Pretoria, South Africa	Neurosyphilis	13	14*a* (54%)
Florindo et al[20]	Lisbon, Portugal	Persons suspected of having early syphilis, based on clinical data and serology	42	14*a* (50%)
Cole et al[16]	Scotland	Infectious syphilis	58	14*d* (76%)
Martin et al[17]	Shanghai, China	Primary syphilis	38	14*f* (79%)
Marra et al[18]	Seattle, WA	Syphilis	66	10 (58%)*

*Other components of type or subtype not reported.

seems to be discriminatory enough with more than 40 strain types identified to date.[11,13,16,18] Molecular surveillance of syphilis might be able to identify clusters associated with specific transmission networks that could be targets of prevention efforts, as has been shown for gonorrhea[23]; subtypes more likely to cause neurosyphilis, as has been shown in a previous study,[18] possibly requiring more intensive treatment or closer follow-up of patients infected with those strains; or new strains in areas with endemic syphilis, indicating bridging of populations or the beginning of an epidemic requiring more intense prevention and control measures. Further research into the molecular epidemiology of syphilis might contribute to making *T. pallidum* subtyping useful for public health practice.

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