BRIEF REPORT

Macrolide Resistance in *Treponema pallidum* in the United States and Ireland

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For decades, syphilis infection has been treated with penicillin, and *Treponema pallidum* has not developed resistance to penicillin. In many countries, the recommended treatment for early syphilis is a single dose of penicillin G benzathine, which maintains bactericidal levels for weeks, killing the slowly metabolizing treponemes. Azithromycin, which has a long tissue half-life and can be administered orally, was found to be effective in the treatment of syphilis in a rabbit model[1] and in small studies in humans.[2-6] Because of its convenience and efficacy, azithromycin is increasingly being used for the treatment of syphilis by clinicians and in disease-control activities in Canada and the United States, although it is not currently recommended by the Centers for Disease Control and Prevention.[7]

We discuss one patient with clinical failure of azithromycin therapy for syphilis, among several cases that have been recognized.[8] We identified a mutation in the 23S ribosomal RNA (rRNA) genes in a specimen of *T. pallidum* obtained from this patient, and we confirmed functional azithromycin resistance in vivo in a strain of *T. pallidum* that contain this mutation. Testing of *T. pallidum* samples obtained at four geographically diverse sites revealed a high frequency of this mutation in clinical specimens.

METHODS

SAMPLES

Swab samples were collected from primary or moist secondary syphilis lesions in patients at the San Francisco Department of Public Health Municipal Sexually Transmitted Disease (STD) Clinic, the Baltimore City Health Department Eastern STD Clinic, and, in Ireland, the Department of Genito-Urinary Medicine and Infectious Diseases, or GUIDE, Clinic, St. James Hospital, Dublin. In Seattle, *T. pallidum* strains recently isolated from blood and cerebrospinal fluid by rabbit inoculation were provided for analysis. We reviewed medical records to obtain demographic and medical information about syphilis treatment. Approval for these studies was obtained from the institutional review board at each site, and written informed consent was obtained from all subjects. Eighteen historical strains of *T. pallidum*, isolated between 1912 and 1987, were propagated by rabbit passage for analysis.

GENE SEQUENCING AND RESTRICTION-DIGESTION ANALYSIS

Initially, samples from two patients in San Francisco in whom clinical failure of azithromycin therapy was suspected were examined with the use of DNA sequencing for mutations in the 23S rRNA gene sequences. For details of the method of sample collection and the molecular analysis, see the Supplementary Appendix (available with the full text of this article at www.nejm.org). For rapid screening of samples for the identified

23S rRNA gene mutation, a polymerase-chain-reaction (PCR)–based restriction-digestion assay was developed. *Mbo*II digestion of purified 628-bp PCR amplicons (representing the portion of the 23S rRNA gene containing the site of the mutation) was performed, and the digestion products were separated with the use of agarose gels. Samples without the mutation show a single band at 628 bp, whereas those with the mutation yield two fragments of 440 and 188 bp. DNA sequencing of eight samples that were identified by the restriction-digestion assay confirmed the mutation.

To ensure that DNA from other bacteria was not being amplified during the PCR and to determine whether the mutation was present in one or both of the 23S rRNA genes of *T. pallidum*, a nested PCR amplification was developed with the use of first-round antisense primers that are *T. pallidum*–specific.

IN VIVO RESISTANCE STUDIES

Four groups of three adult male New Zealand white rabbits were infected intradermally with either the *T. pallidum* Nichols strain (which is susceptible to azithromycin) or the Street 14 strain (which contains the identified 23S rRNA mutation), as previously described.[1] The study included untreated controls. Treatment consisted of penicillin G benzathine at 200,000 U per rabbit (equivalent to 4.8 MU wt/wt in humans) given intramuscularly as a single dose, azithromycin at 45 mg per day (equivalent to 1 g per day wt/wt in humans) given orally once daily for two weeks, or erythromycin at 90 mg per day (equivalent to 2 g per day wt/wt in humans) given orally four times daily for two weeks. Material was aspirated from one lesion on each rabbit daily for a period of two weeks and examined with the use of dark-field microscopy for presence of *T. pallidum*. At least 100 fields were examined, and the microscopists were blinded to the treatment assignments. Venereal Disease Research Laboratory (VDRL) titers were determined six weeks after the initiation of therapy.

RESULTS

CLINICAL FAILURE OF AZITHROMYCIN THERAPY IN A PATIENT IN SAN FRANCISCO

In July 2003, a physician in a local emergency room saw a 33-year-old man with human immunodeficiency virus (HIV) infection who reported that he had had a nontender penile ulcer for one week. He was treated with azithromycin at a dose of 2 g orally, and he independently took an additional 1 g of azithromycin the next day. One day after taking the additional dose, he came to the San Francisco Municipal STD Clinic for follow-up. He reported having had insertive oral and anal intercourse with a single partner during the past 90 days. He had a history of genital herpes and urethral gonorrhea, and the results of previous VDRL testing had been nonreactive.

On physical examination, he had an indurated, nontender ulcer 1 cm in diameter on the glans penis and left inguinal lymphadenopathy. The rapid plasma reagin test was reactive, but on dark-field microscopical examination, the lesion was negative. He was given a diagnosis of primary syphilis and was considered to have received adequate treatment. The patient returned three days later with a persistent ulcer that was dark-field–positive for *T. pallidum*. The serum VDRL test and *T. pallidum* particle-agglutination test were reactive. Azithromycin treatment failure was diagnosed, and the patient was treated with penicillin G benzathine at a dose of 2.4 mU intramuscularly. The patient returned two weeks later, and the lesion had resolved; the follow-up VDRL test at three months was nonreactive.

IDENTIFICATION OF THE MUTATION IN THE 23S rRNA GENES

Sequencing of the 23S rRNA genes in the samples from two patients in San Francisco with clinical az-

T. pallidum Strain	23S rRNA Gene	Mutation
Nichols	TAGACGG**A**AAGACCCC	Wild type
Street 14	TAGACGG**G**AAGACCCC	A→G
CA 42	TAGACGG**G**AAGACCCC	A→G
CA 61	TAGACGG**G**AAGACCCC	A→G
Dub 21	TAGACGG**G**AAGACCCC	A→G
Dub 25	TAGACGG**G**AAGACCCC	A→G
Dub 58	TAGACGG**G**AAGACCCC	A→G
Dub 49	TAGACGG**G**AAGACCCC	A→G
UW 133	TAGACGG**G**AAGACCCC	A→G
UW 157	TAGACGG**G**AAGACCCC	A→G

Figure 1. Sequence Analysis of the 23S rRNA Gene in Selected Strains of *Treponema pallidum*.

Only the portion of the sequence of the 23S rRNA gene containing the mutation is shown; the A→G mutation (indicated in bold type) is located at the position cognate to A2058 in the 23S rRNA gene in *Escherichia coli*. CA denotes San Francisco, Dub Dublin, and UW Seattle.

ithromycin treatment failure revealed A→G mutations at the position cognate to A2058 in the *Escherichia coli* 23S rRNA gene, a mutation identical with the mutation previously associated with macrolide resistance in the Street 14 strain of *T. pallidum*.[9] Subsequently, this mutation was confirmed with the use of DNA sequencing in four other specimens obtained in Dublin and two obtained in Seattle (Fig. 1).

SCREENING OF SAMPLES FROM MULTIPLE GEOGRAPHIC SITES

A convenience sample of 114 specimens obtained from San Francisco, Seattle, Baltimore, and Dublin were screened with the use of the restriction-digestion assay (Fig. 2). As shown in Table 1, the mutation was identified in 15 of 17 samples (88 percent) from Dublin, 12 of 55 samples (22 percent) from San Francisco, 3 of 23 samples (13 percent) from Seattle, and 2 of 19 samples (11 percent) from Baltimore. The proportion of San Francisco samples that contained the mutation was higher among recent cases: 1 of 25 (4 percent) from the period 1999 through 2002, as compared with 11 of 30 (37 percent) from 2003. In all cases, the mutation was confirmed in both copies of the 23S rRNA gene with the use of nested PCR. In a survey of 18 historical isolates of *T. pallidum* collected between 1912 and 1987, only the Street 14 strain contained the mutation.

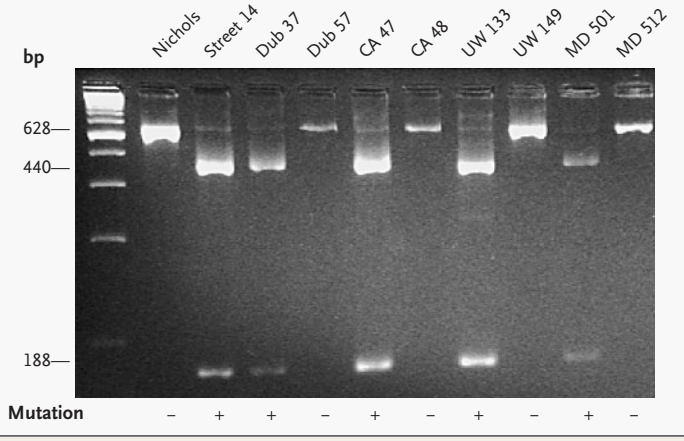

Figure 2. Restriction-Digestion Analysis of the 23S rRNA Gene Amplicon from Two Characterized Strains of *T. pallidum* (Nichols and Street 14) and from *T. pallidum* Samples from Dublin, San Francisco, Seattle, and Baltimore.

Fragments at 440 and 188 bp indicate the presence of the mutation, signified by the plus (+) sign; the intact amplicon at 628 bp indicates the absence of the mutation, signified by the minus (–) sign. Dub denotes Dublin, CA San Francisco, UW Seattle, and MD Baltimore. The first lane shows the molecular-size standard.

CONFIRMATION OF AZITHROMYCIN RESISTANCE IN VIVO

Rabbit infections with both the Nichols and the Street 14 strains were susceptible to penicillin G benzathine, whereas only the Street 14 strain (containing the mutation) was resistant to treatment with azithromycin or erythromycin (Table 2). In all rabbits infected with the Street 14 strain *T. pallidum* was detectable in the lesions after two weeks of treatment with azithromycin or erythromycin, whereas in rabbits infected with the Nichols strain there was no detectable *T. pallidum* in the lesions at the end of macrolide therapy (Table 2). Treatment with penicillin G benzathine resulted in the rapid clearance of *T. pallidum* from the lesions resulting from both strains. The VDRL titers measured in all rabbits after treatment with penicillin G benzathine, and in Nichols-infected rabbits that were treated with azithromycin and erythromycin, were significantly lower than those in untreated control rabbits. In contrast, the VDRL titers measured in Street 14–infected rabbits that were treated with erythromycin or azithromycin were not different from those in untreated controls.

DISCUSSION

The drug of choice for the treatment of syphilis is penicillin, and to date there have been no documented reports of penicillin resistance in *T. pallidum* strains. Owing to the discomfort to the patient of intramuscular administration of penicillin G benzathine, many clinicians have sought alternative oral treatments, including tetracycline, doxycycline, and erythromycin. The adverse effects and difficult dosing schedules required by these drugs can result in poor compliance and therefore higher rates of treatment failure[10]; consequently, the tetracyclines and macrolides are alternatives only for patients who cannot receive penicillin.[7] The development of azithromycin, a macrolide antibiotic with a long tissue half-life, provided the possibility of an effective oral therapy for syphilis. Previous studies have suggested that azithromycin is as effective as penicillin G benzathine in the rabbit model of primary syphilis[1] and in humans with incubating,[4] early,[2,3,6] or seropositive[5] syphilis.

Syphilis is again epidemic in many cities in the United States, the British Isles, and Europe, particularly among men who have sex with men,[11-20] many of whom are HIV-infected. A major challenge in controlling these outbreaks of syphilis is the large

number of anonymous and unnamed sex partners. Notification of partners and epidemiologic treatment have been the keystone of successful control of syphilis for many decades, but they have had a limited effect on the current outbreaks. In response, some clinicians and public health officials have expanded epidemiologic treatment by encouraging patients infected with syphilis to provide their sexual partners with azithromycin as preventive therapy.[16,21]

To investigate apparent failures of azithromycin treatment among patients with syphilis in San Francisco,[8] we initiated a study to identify the molecular mechanism involved. Until now, the single documented *T. pallidum* isolate with macrolide resistance was the Street 14 strain.[22] Resistance of this strain to macrolides was confirmed in vitro,[23,24] and the mutation conferring the resistance was found to be an A→G mutation at the position cognate to A2058 in the *E. coli* 23S rRNA gene.[9] We identified the identical mutation with the use of DNA sequencing or a rapid PCR-based restriction-digestion assay in 32 of 114 samples of *T. pallidum* from four geographically diverse sites (28 percent).

In the San Francisco samples, the proportion containing the mutation was higher for samples obtained during 2003 than for those obtained in the period from 1999 through 2002, suggesting that a mutated strain has either recently been introduced into a sexual network or has been selected for among persons who engage in high-risk behavior. In Dublin, a very high proportion (88 percent) of samples contained the mutation, suggesting the introduction of a mutant strain that spread rapidly within a defined sexual network. The rapid spread of a particular strain of *T. pallidum* might occur within a sexual network even without antibiotic selection. However, macrolides are frequently used for the treatment of respiratory tract infections, and, specifically, azithromycin is used for treatment of *Chlamydia trachomatis* and for prophylaxis against infection with *Mycobacterium avium* complex in persons with the acquired immunodeficiency syndrome. It is possible that widespread use of macrolides, including azithromycin, in persons who engage in high-risk behavior might have selected for strains of *T. pallidum* that contain this mutation.

The identification of macrolide resistance in *T. pallidum* samples from several geographic areas suggests the need for caution in the use of azithromycin therapy for syphilis. We recognize, however, that our specimens were obtained at selected STD

Table 1. The Presence of the 23S rRNA Gene Mutation in *T. pallidum* Samples Collected from Sites in the United States and Ireland from 1912 through 2003.

Geographic Site	Date Sample Collected	Samples with Mutation/ Total Amplifiable Samples
		no./total no. (%)
San Francisco	1999–2002 2003	1/25 (4) 11/30 (37)
Seattle	2001–2003	3/23 (13)
Baltimore	1998–2000	2/19 (11)
Dublin	2002	15/17 (88)
Historical strains from multiple locations	1912–1987	1/18 (6)

Table 2. Efficacy of Therapy for Primary *T. pallidum* Infection in the Rabbit Model, According to Strain and Type of Therapy.*

Outcome	Nichols Strain				Street 14 Strain			
	Untreated Control	Penicillin G Benzathine	Erythromycin	Azithromycin	Untreated Control	Penicillin G Benzathine	Erythromycin	Azithromycin
No. of animals with DF-positive lesions at completion of therapy/total no. studied	3/3	0/3	0/3	0/3	3/3	0/3	3/3	3/3
Days to DF negativity — median (range)	>14	1 (1)	3 (3–4)	3 (2–3)	>14	1 (1)	>14	>14
VDRL titer six weeks after initiation of therapy — median (range)	8 (4–8)	0 (0–WR)†	2 (2–4)†	1 (1)†	8 (4–8)	0 (0)†	4 (4–8)	1 (0–8)

* DF denotes dark-field microscopical examination of lesions for presence of *T. pallidum*, VDRL Venereal Disease Research Laboratory, and WR weakly reactive.
† $P<0.05$ for the comparison with untreated controls for each strain, by means of analysis of variance.

clinics in the United States and Ireland, and that these clinics may not represent the true prevalence of macrolide resistance in currently circulating strains of *T. pallidum* elsewhere. For example, a multicenter trial of a single dose (2 g) of azithromycin for early syphilis is under way in Madagascar and in the United States in Birmingham, Alabama; Chapel Hill, North Carolina; New Orleans; and Indianapolis. To date, no clinical failures have been observed in the approximately 100 subjects randomly assigned to receive azithromycin and observed for at least six months (Hook EW III: personal communication).

There are several potentially important differences between the patients who were enrolled in this multicenter clinical trial and those in our study. First, no subjects in the multicenter clinical trial are infected with HIV, whereas many of our subjects are infected with HIV. Second, the race or ethnic group and the demographic characteristics of the subjects in the multicenter clinical trial are very different from those of most of our subjects, and approximately half of those enrolled in the multicenter trial are from Madagascar. Third, there are likely to be substantial geographic differences in the distribution of *T. pallidum* strains.

The recommended treatment for syphilis infection is penicillin G benzathine. Our data suggest that, in geographic regions where macrolide resistance may be relatively high, the use of azithromycin for treating syphilis infection should be avoided. In addition, persons treated with nonpenicillin regimens such as azithromycin should be closely followed and instructed to return to the hospital or clinic if symptoms persist. Further studies should be conducted to determine the prevalence of *T. pallidum* strains with the macrolide-resistance mutation and to evaluate the efficacy of azithromycin treatment in syphilis infection.

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