ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2000, p. 806–807 Vol. 44, No. 3
0066-4804/00/$04.00+0
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Letter to the Editor

A Point Mutation Associated with Bacterial Macrolide Resistance Is Present in Both 23S rRNA Genes of an Erythromycin-Resistant *Treponema pallidum* Clinical Isolate

Treponema pallidum subsp. *pallidum* (*T. pallidum*) is the noncultivable agent of syphilis, a sexually transmitted disease that is a risk factor for HIV infection (9). Penicillin is the preferred drug for treatment of syphilis (1). Alternative antibiotics for nonpregnant penicillin-allergic patients with primary or secondary syphilis include doxycycline, tetracycline, or erythromycin. Erythromycin treatment failures have been documented in patients with primary or secondary syphilis and infants with congenital syphilis (2, 3, 5). We previously demonstrated high-level erythromycin resistance (Erm^r) in a *T. pallidum* clinical isolate (Street strain 14) obtained from a syphilis patient who failed erythromycin therapy (11). Macrolide resistance usually results from target site alteration due to methylation or mutations in the peptidyltransferase region of 23S rRNA. Mutations of the adenine (A) residue cognate to position A2058 or A2059 in the *Escherichia coli* 23S rRNA gene are associated with macrolide resistance in bacteria that contain one or two copies of these genes (6–8, 13). This observation prompted us to analyze the corresponding regions of the two 23S rRNA genes of *T. pallidum* Street strain 14 (Erm^r); *T. pallidum* Nichols strain, an erythromycin-sensitive (Erm^s) control (11); and the closely related yaws agent, *T. pallidum* subsp. *pertenue* (*T. pertenue*) Gauthier strain (Erm^s) (11).

T. pallidum strains were grown by testicular cultivation in rabbits, and genomic DNA was extracted as previously described (11, 12). *T. pertenue* genomic DNA was provided by A. Centurion-Lara and S. Lukehart. Both copies of the 23S rRNA genes were PCR amplified from treponemal genomic DNA (Expand Long Template PCR System; Boehringer-Mannheim, Indianapolis, Ind.). Oligonucleotide primers were designed based on sequence data from the *T. pallidum* Nichols genome sequencing project (GenBank accession no. AE001204 and AE001208) (4). Gel-purified PCR amplicons were cloned (pGEM-T Easy vector; Promega Corp., Madison, Wis.), and both strands of the inserts were sequenced at the University of North Carolina, Chapel Hill, Automated DNA Sequencing Facility as previously described (12).

TABLE 1. Comparison of the 23S rRNA gene sequences of *T. pallidum* Nichols and Street strain 14 and *T. pertenue* in the region containing the cognate to *E. coli* rDNA A2058

Organism	23S rRNA[a]	Susceptibility to erythromycin[b]	Mutation[c]
T. pallidum Nichols	TAGTTAGACGGAAAGACCCC	S	—
T. pallidum SS14	TAGTTAGACGGGAAGACCCC	R	A→G
T. pertenue Gauthier	TAGTTAGACGGAAAGACCCC	S	—

[a] Only the relevant portion of the 692-bp region is shown. Sequences of the two 23S rRNA genes for each respective treponeme are identical. The transition mutation in the *T. pallidum* Street strain 14 (SS14) 23S rRNA gene sequence cognate to *E. coli* 23S rDNA A2058 is underlined.

[b] Erythromycin susceptibility (S) or resistance (R) was determined previously in an in vitro assay (11).

[c] —, wild type.

A 692-bp region (representing 133 nucleotides 5′ and 558 nucleotides 3′ of the position cognate to A2058 in the *E. coli* 23S rRNA gene) was analyzed for *T. pallidum* and *T. pertenue*. The nucleotide sequences of this region of both *T. pallidum* Nichols strain 23S rRNA genes were identical to those of the Nichols strain in GenBank (4). Additionally, the sequences of the same 692-bp region of both *T. pertenue* Gauthier strain 23S rRNA genes were identical to those of the *T. pallidum* Nichols strain. Interestingly, the sequences of the 692-bp region of both *T. pallidum* Street strain 14 23S rRNA genes were identical to those of *T. pallidum* Nichols strain except that the Street strain 14 sequences contained a guanine (G) at the position cognate to A2058 (Table 1). The A-to-G transition mutation was present in all clones sequenced for both Street strain 14 23S rRNA genes. The identical mutation was also present when the Street strain 14 23S rRNA genes were PCR amplified and directly sequenced.

Since rRNA methylase genes do not appear to be present in *T. pallidum* Street strain 14 (L. Stamm, unpublished data), we propose that the A-to-G transition mutations in both 23S rRNA genes of this spirochete are responsible for its high-level resistance to erythromycin and related macrolides (roxithromycin [11] and azithromycin [10]). Similar point mutations in the 23S rRNA genes of other *T. pallidum* Street strains may account for erythromycin treatment failures observed in syphilis patients who have complied with their therapeutic regimen. Our results also demonstrate the utility of the *T. pallidum* Nichols strain complete genome sequence for investigating the genetic basis of antimicrobial resistance in *T. pallidum*.

Nucleotide sequence accession numbers. The sequences of the 692-bp region of the 23S rRNA genes of *T. pallidum* Nichols strain and Street strain 14 and *T. pertenue* Gauthier strain have been deposited in GenBank under accession numbers AF200367, AF200365, and AF200366, respectively.

This research was supported by National Institutes of Health grant AI31496.

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