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 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’) 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 2058 or 2059 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’); T. pallidum Nichols strain, an erythromycin-sensitive (Erm’) control (11); and the closely related yaws agent, T. pertenue subsp. pertenue (T. pertenue) Gauthier strain (Erm’) (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).

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 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.

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REFERENCES


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