Two Mutations Associated with Macrolide Resistance in *Treponema pallidum* in Shandong, China

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We read with interest the report by Wu et al. (1) showing that none of the 211 *Treponema pallidum* strains examined exhibited 23S rRNA mutations (i.e., A2058G and A2059G) associated with resistance to macrolides. Their finding suggests that azithromycin remains an alternative treatment for early syphilis in nonpregnant patients in Taiwan who are intolerant of penicillins. This result is different from our latest findings in mainland China.

Both the A2058G and A2059G mutations in the 23S rRNA gene of *T. pallidum* are reported to be associated with macrolide resistance (2, 3). The presence of the A2058G mutation in *T. pallidum* has been documented among strains surveyed from different regions of the world, namely, 100% in strains from Shanghai, China (4), 93.1% for Dublin, United Kingdom (5), 66.6% for London, United Kingdom (6), 62% for Seattle, WA (7), 56% for San Francisco, CA (8), 28.6% for Canada (9), and 1% for Southern Africa (10). Recently, strains harboring the A2059G mutation have also been observed. The proportion of *T. pallidum* strains with the A2059G mutation was reported to be 13.2% in the United States (11). Grimes et al. (7) observed that 10% of the *T. pallidum* strains obtained from men who have sex with men (MSM) in Seattle, WA, during the past decade harbored the A2059G mutation. In London, United Kingdom, 5.6% of the *T. pallidum* specimen examined harbored the A2059G mutation (6). So far, no one has found the A2059G mutation in China. Reports from other countries (e.g., Madagascar, Tanzania, and Uganda) have not described the presence of 23S rRNA mutations (11, 12, 13, 14).

We performed this study to determine the prevalences of the A2058G and A2059G mutations in *T. pallidum* isolated from patients in Shandong, China. The study was approved by the human medical and ethics committee of Shandong Provincial Hospital for Skin Diseases. Specimens (n = 73) were collected from anogenital lesions from HIV-negative patients presenting with primary and secondary syphilis at Shandong Provincial Hospital for Skin Diseases between 2010 and 2012. DNA samples from 66 specimens that tested positive for *T. pallidum* by real-time PCR targeting the *polA* gene were identified (5), and the 23S rRNA genes were amplified by nested PCR. The primer sequences were as follows: 23srRNA-outer-F, GTACCGGACAACGGACACAG; 23srRNA-outer-R, GCGCGAAACCTCTTTTAC; 23srRNA-inner-F, TGACCCTGCTCTTGTTGAG; 23srRNA-inner-R, GGATCACCAGGCAAAGGTTA. PCR amplification was performed under the following cycling conditions: 95°C (3 min), 95°C (40 s), 55°C (1 min), 72°C (1 min), and 72°C (5 min) for 35 cycles using an ABI Prism 7500 Fast sequence detection system. The second step of the nested PCR was performed under the same conditions. Sequencing of the 23S rRNA gene using an ABI Prism 3130xl DNA analyzer identified the A2058G and A2059G mutations. The percentage of specimens with either the A2058G or A2059G mutation was 100% (66/66). The A2058G mutation was detected in 61/66 (92.4%) specimens, while the A2059G mutation was detected in 5/66 (7.6%) specimens. The latter mutation was detected in specimens collected in 2011 (3/24) and in 2012 (2/38), suggesting that it has been present for at least 3 years. This report identifies, for the first time in China, the existence of strains containing the A2059G mutation. The extremely high prevalence of macrolide-resistant *T. pallidum* in Shandong may be related to the extensive use of the antibiotics in clinical treatment. Macrolides have been widely used for the treatment of respiratory diseases, skin infections, and sexually transmitted disease infections in China, providing the background for the development of macrolide resistance.

In summary, we found a high prevalence of azithromycin-resistant *T. pallidum* in Shandong, which is consistent with the findings in different areas in China (4, 15) and suggested that currently circulating *T. pallidum* strains in Taiwan may be unrelated to those in mainland China. We report the presence of two mutations in the 23S rRNA gene of *T. pallidum*, which further expands our knowledge of the geographical distribution of these strains.

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**REFERENCES**

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