The development of doxycycline resistance by *T. pallidum* could have devastating consequences for the control of syphilis and is being closely monitored by clinicians and scientists. We are encouraged by increasing genomic surveillance of *T. pallidum*, providing a mechanism for early detection of the emergence of resistance-associated variants.

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In Response: On March 5, 2026, Drs. Lieberman and Beale contacted us concerning the identification of a heterozygous allele at position G966T (by *E. coli* reference numbering, G969T by *T. pallidum* reference numbering) of the 16S rRNA gene of *Treponema pallidum* subspecies *pallidum* (*1*). They surmised that our identification of the allele may have been caused by either genome misassemblies or contamination with sequences from other *Trepomena* species (*2*). In response to these reproducibility concerns, we undertook a separate analysis using an independent analytical process from our original work (*1*).

Our reanalysis focused on the sequencing libraries of Beale et al. (*3*–*5*) and applied a mapping-based approach, aligning reads to the 16S rRNA gene of the *T. pallidum* reference genome (NC_021490.2:231255–232803). That reanalysis concluded that the previously identified heterozygous allele was indeed the result of contamination. Accordingly, Table 4 and text associated with the heterozygous single-nucleotide polymorphism have been removed from the original article. In addition, an extra paragraph has been added to the Appendix detailing the additional analyses.

Of note, the overall findings of our study remain unaffected. We still report the sequencing and assembly of 15 *T. pallidum* subsp. *pallidum* genomes from Canada. This revision strengthens the clarity of our comparative genomics analysis and simplifies the identification of single-nucleotide polymorphisms related to putative doxycycline resistance and underscore the importance of accounting for the presence of two 16S rRNA gene copies in *T. pallidum*. This study continues to represent a vital step in establishing a genomic baseline for monitoring circulating *T. pallidum* subsp. *pallidum* lineages in Canada.

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