

Antibiotic resistance in *Treponema pallidum* subsp. *pallidum*, the syphilis agent

Resistência a antibióticos do Treponema pallidum subsp. pallidum, o agente da sífilis

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Syphilis, caused by *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), remains a global health problem, with a rising incidence worldwide^(1,2) and significant challenges in disease control in both low- and middle-income countries and in high-income nations. Once syphilis is diagnosed, the recommended treatment is penicillin G in different formulations and dosages, depending on disease stage and involvement of the central nervous system⁽³⁾. In addition to penicillin G, other natural or synthetic β -lactams have shown efficacy against *T. pallidum* in pre-clinical⁽⁴⁾ and clinical studies. Amoxicillin, for example, was effective for syphilis treatment^(5,6). Ceftriaxone, a third-generation β -lactam cephalosporin, is listed as an alternative to penicillin G for early syphilis, and data support its efficacy for neurosyphilis as well⁽⁷⁻⁹⁾. Promising results are also available on the use of another third-generation cephalosporin, cefixime, to treat early syphilis^(10,11).

The reliance on β -lactam antibiotics for syphilis treatment is strongly justified by the low minimal inhibitory concentration (MIC) exhibited by these antibiotics when tested against *T. pallidum*^(4,12) and, most importantly, by the lack of evidence that genetic resistance ever developed to this class of antibiotics during the 80 years of uninterrupted use to treat syphilis. One possible reason why *T. pallidum* has not developed genetic resistance to β -lactams, which is to be found in the lack of plasmids or other mobile elements in the pathogen's genome, which makes the acquisition and retention of episomes containing genes coding for β -lactamases or other resistance genes quite unlikely. However, because *T. pallidum* is naturally competent for transformation⁽¹³⁾, the uptake of environmental deoxyribonucleic acid (DNA) fragments carrying β -lactamase genes could occur. In this scenario, one could speculate that these exogenous DNA fragments lacked sufficient homology to *T. pallidum* DNA to recombine with and integrate into the chromosome. Additionally, even if these recombination events occurred, their outcomes might not be advantageous to the pathogen, as a significant proportion of its genes are likely to be essential. During its evolution to become an obligate human pathogen, *T. pallidum* has undergone genomic reduction⁽¹⁴⁾ that led to the elimination of genes encoding many metabolic and biosynthetic pathways whose products are now provided by the infected host, while retaining other essential genes and virulence factors. One could postulate that altering the sequence or expression pattern of many *T. pallidum* genes in its minimal genome may result in a non-viable phenotype, which limits the acquisition

of new genes and, hence, explains the monomorphic nature of the *T. pallidum* genome.

Regarding susceptibility to β -lactams, investigators have postulated that point mutations in *T. pallidum* genes encoding penicillin-binding proteins (PBPs) could confer resistance or limit the efficacy of these compounds. This mechanism would bypass the need for environmental DNA acquisition and risky genomic rearrangements. An early study identified five single-nucleotide polymorphisms in three PBPs, and the authors hypothesized that these changes could translate into structural modifications able to reduce the pathogen's susceptibility to β -lactams⁽¹⁵⁾. The study, however, suffered from the unavailability of viable strains to assess the contributions of these polymorphisms *in vivo* or *in vitro*. Another study reported that one of the *T. pallidum* PBPs, the immunodominant 47 kDa lipoprotein (TpN47), exhibits β -lactamase activity *in vitro*⁽¹⁶⁾. However, the authors also showed that TpN47 activity was inhibited by hydrolyzed penicillin, explaining why the syphilis agent remains susceptible to penicillin. The authors, nonetheless, hypothesized that mutations capable of bypassing this product inhibition phenomenon could arise, although no experimental evidence for those is currently reported.

In 2025, two laboratory-derived mutant *T. pallidum* strains carrying the A1873G mutation (inducing the amino acid change M625V) in the PBP-encoding *tp0705* gene were reported to be less susceptible to ceftriaxone and penicillin G at low concentrations of these antibiotics⁽¹³⁾, although with very modest absolute effect sizes. We recently tested the *in vitro* susceptibility to penicillin G and ceftriaxone of three *T. pallidum* clinical isolates (UW244B, UW249B, and UW330B), each carrying a distinct variant of the Tp0705 protein, including the one studied in⁽¹³⁾, due to polymorphisms at positions 1516, 1873, and 2122 of the gene. The goal of this study was to assess whether these naturally occurring Tp0705 variants would alter susceptibility to β -lactams in these strains, which have identical PBPs, except for Tp0705. These *T. pallidum* isolates, however, were all equally susceptible to penicillin G and ceftriaxone⁽¹⁷⁾.

Overall, even in the absence of concrete evidence for resistance to β -lactams in the syphilis agent, one should assume that resistance could eventually emerge. Although reports of penicillin treatment failures remain relatively rare, investigators should do their best to obtain treponema-containing samples from patients who allegedly failed β -lactam treatment for further studies. At the same time, given that low concentrations of β -lactams are not treponemicidal, an effort should be made to determine the post-administration level of these antibiotics attained in tissues and bodily compartments known to harbor *T. pallidum* during infection so that adequate concentrations can be achieved and maintained to ensure a cure.

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In contrast to β -lactams, resistance to macrolides developed and spread rapidly when oral azithromycin was introduced for early syphilis treatment, to the point that virtually all *T. pallidum* strains circulating in North America are macrolide-resistant⁽¹⁸⁾. In this pathogen, macrolide resistance is associated with either the A2058G or the A2059G mutations in the 23S rRNA gene⁽¹⁹⁾. Currently, based on available molecular typing data, the percentage of macrolide-resistant *T. pallidum* strains varies by country and is likely to reflect the extent of macrolide use within each healthcare system. Even in countries where such a percentage is not near 100%, providers should avoid prescribing azithromycin for early syphilis and assume that their patients are infected with a macrolide-resistant strain to minimize the risk of treatment failure, disease progression, and selection of these resistance-associated mutations.

Doxycycline and tetracycline have been used for years and are effective for early and latent syphilis⁽³⁾. Whether *T. pallidum* can develop resistance to doxycycline and tetracyclines is also the topic of an ongoing debate. Tetracyclines block protein synthesis by binding to the 16S rRNA within the 30S ribosomal subunit⁽²⁰⁾. Past studies demonstrated that doxycycline had a success rate comparable to penicillin for early syphilis⁽²¹⁻²⁴⁾. In other bacterial pathogens, tetracycline or doxycycline resistance can be mediated by the acquisition and expression of efflux systems, ribosomal protection proteins, or drug-inactivating enzymes⁽²⁰⁾. In a recent report, authors claimed to have identified the efflux pump-encoding gene *tetB* in 15 samples from syphilitic lesions⁽²⁵⁾; however, the detected resistance gene might also have been from other bacteria also present in the sampled area. Mutations in ribosomal protein-encoding genes or in the 965-967 triplet of the 16S rRNA gene have also been associated with tetracycline resistance in other pathogens⁽²⁰⁾. Whether those changes would confer tetracycline resistance if present in the *T. pallidum* 16S rRNA gene is unclear.

While there are no reliable reports of a tetracycline-resistant *T. pallidum* strain, concerns persist regarding the possible emergence of doxycycline resistance following the widespread adoption of doxycycline post-exposure prophylaxis. The increased and intermittent use of doxycycline among individuals at risk for sexually transmitted infections could favor selection of doxycycline resistance in *T. pallidum* in the same way macrolide use selected for resistance-associated mutations in the syphilis agent. In a recent *in vitro* study we authored, *T. pallidum* was intermittently exposed to doxycycline for over 7 months and then continuously for 10 weeks. Following these prolonged exposures, genotypic and phenotypic analyses found no doxycycline resistance, although the tested strain appeared to exhibit some limited tolerance to low concentrations of doxycycline toward the end of the exposure experiment, which, however, did not result in any change in the drug's MIC for *T. pallidum*⁽²⁶⁾.

In conclusion, although *bona fide* resistance to antibiotics in *T. pallidum* has been described so far only for macrolides, it has never been reported for other classes of antibiotics used to treat syphilis. Given the foreseeable increase in the use of prophylactic doxycycline for sexually transmitted bacterial infections and the fact that approved therapeutics for syphilis remain limited, heightened surveillance and alertness will be pivotal for this pathogen in the years to come. Thanks to advances in the *in vitro* propagation of *T. pallidum*⁽⁴⁾ and a deeper understanding of *T. pallidum* genomics⁽²⁷⁾, research into alternative effective medications for syphilis is ongoing

and will likely lead to new treatment options in the future, which will further mitigate the threat of antibiotic resistance in this pathogen.

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