Article

Not peer-reviewed version

# Tetracycline Resistance Genes in *Treponema* spp.- an Analysis of 4355 Spirochaetales Genomes

Sheeba Manoharan-Basil *, Zina Gestels, Said Abdellati, Irith De Baetselier, Thibaut Vanbaelen, Chris Kenyon

Posted Date: 11 March 2024

doi: 10.20944/preprints202403.0563.v1

Keywords: Syphilis; Tetracycline resistance; *Treponema pallidum*

 doi:10.20944/preprints202403.0563.v1



*Article*

# Tetracycline Resistance Genes in *Treponema* spp.—An Analysis of 4355 Spirochaetales Genomes

**Sheeba Santhini Manoharan-Basil [1,*,†], Zina Gestels [1], Said Abdellati [1], Irith De Baetselier [2], Thibaut Vanbaelen [1] and Chris Kenyon [1,3,†]**

[1] Department of Clinical Sciences, Institute of Tropical Medicine Antwerp, 2000 Antwerp, Belgium
[2] Clinical and Reference Laboratory, Department of Clinical Sciences, Institute of Tropical Medicine, 2000 Antwerp, Belgium
[3] Department of Medicine, University of Cape Town, Cape Town 7700, South Africa
* Corresponding author: Department of Clinical Sciences, Institute of Tropical Medicine Antwerp, 2000 Antwerp, Belgium. Email: sbasil@itg.be
† These authors contributed equally to this work.

**Abstract: Background:** The resurgence of syphilis, primarily in MSM populations, has necessitated novel prophylactic strategies, such as the use of doxycycline post-exposure prophylaxis (doxy-PEP). However, the potential for increased doxycycline use to select for tetracycline resistance represents significant challenges in managing this sexually transmitted infection. This study aims to identify chromosomal mutations associated with tetracycline resistance in Spirochaetales to inform treatment strategies and surveillance measures. **Methods:** Whole genome sequences (WGS) from the Spirochaetales order, including 4,355 genomes, were analyzed for the presence of mutations in 16S rRNA and non-synonymous mutations in the *rpsC* and *rpsJ* genes. The study utilized WGS from GenBank® and sequence data from the PubMLST *Treponema pallidum* isolate collection. Genetic resistance to tetracycline was detected using a combination of BLASTN searches and gene-gene analysis. **Results:** A transition mutation TGA to TGG at positions 965-967 in the 16S rRNA gene was detected in 5.6% of Treponema spp. and 3.97% of Spirochaeta spp. genomes. The *rpsJ* gene exhibited a V57G amino acid substitution across a significant subset of *Treponema* spp. Notably, the V57K mutation was common in Spirochaeta spp. The *rpsC* gene had the H178Q mutation and was found to be present in the Spirochaetales bacterium. **Conclusion:** The identification of mutations associated with tetracycline resistance in Spirochaetales provides a foundation for the development of rapid molecular diagnostics and guides the clinical selection of antibiotics for syphilis treatment. This study underscores the complexity of antibiotic resistance mechanisms and the critical importance of surveillance in the era of antibiotic prophylaxis for STI management.

**Keywords:** syphilis; tetracycline resistance; *Treponema pallidum*

## Introduction

The incidence of bacterial STIs in general, but syphilis in particular, has recently increased dramatically in a number of countries, including Belgium [1]. These increases have led to calls for novel measures to reverse this trend. One of the most promising interventions is the ingestion of doxycycline after condomless sex (Doxycycline post-exposure prophylaxis or doxy-PEP), which has been shown to reduce the incidence of syphilis, chlamydia and possibly gonorrhoea in men who have sex with men (MSM) [2–4]. These findings have led a number of organizations, such as the Centers for Disease Control and Prevention (CDC) and the European AIDS Clinicians Society (EACS), to promote the use of doxy-PEP in sub-populations of MSM [5]. We have calculated that the introduction of doxy-PEP in Belgian HIV-pre-exposure prophylaxis (PrEP) cohorts would increase the consumption of doxycycline by up to 88-fold [6]. This intense consumption of doxycycline could induce and select for antimicrobial resistance (AMR) to tetracyclines and other antimicrobials in a range of bacterial species [7–9]. Doxycycline is a vital backup medication for individuals who cannot receive intramuscular penicillin due to frequent stock-outs, silicone implants or penicillin allergy [10].

 doi:10.20944/preprints202403.0563.v1



If doxy-PEP were to induce tetracycline resistance in *Treponema pallidum* subsp. *pallidum*, the causative agent of syphilis, this would have far-reaching consequences.

Previous genotypic assessments of *T. pallidum* have not found mutations that have been predicted to cause tetracycline resistance [11, 12]. One exception is a study from China that detected the *tetB* gene directly from 15/171 syphilitic lesions via PCR amplification [13]. Concerns have, however, been raised that the *tetB* genes detected represented contamination from other bacteria present in these clinical samples [14]. Because *T. pallidum* is not known to contain any mobile elements, point mutations in specific regions of the gene encoding the 16S rRNA or specific ribosomal proteins would be the most plausible pathway to tetracycline resistance [12, 15]. Furthermore, A2058G and A2059G mutations in *T. pallidum's* 23S rRNA that confer resistance to macrolides have arisen independently in multiple lineages of *T. pallidum* and spread to attain prevalences close to 100% [16, 17]. The 16S and 23S rRNA are part of the same single-copy operon in *T. pallidum*, and thus, point mutations are possible in the 16S rRNA gene.

*T. pallidum* is challenging to culture *in vitro*, and thus far, a single study has established that the doxycycline MICs of the four strains assessed were between 0.06mg/L to 0.1mg/L [18]. No study has attempted to induce tetracycline resistance *in vitro* or *in vivo*. For such studies and for the evaluation of suspected tetracycline resistance in human infections, it would be useful to know which resistance mutations are possible based on what resistance-associated mutations occur in related bacterial species. This study assesses the occurrence and prevalence of tetracycline resistance associated with chromosomal mutations in *Spirochetales*. The *Spirochetales* order contains three families: *Spirochaetaceae, Brachyspiraceae,* and *Leptospiraceae*. Previous studies have found that mutations in 16S, *rpsC* and *rpsJ* have been shown to be associated with tetracycline susceptibility in several other spirochaetes, such as *Borrelia* spp., and *Leptospira* spp. [19–21].

## Materials and Methods

### *Dataset for Analysis*

The whole genome sequence (WGS) used in this study was acquired from GenBank®. This collection included 4,355 genomes from the order Spirochaetales (NCBI Taxonomy ID: 136). Additionally, the study incorporated 16S sequence data from PubMLST *Treponema pallidum* isolate collection. This collection included 613 sequences.

### *Detection of genetic resistance to tetracycline*

WGS were screened for tetracycline resistance, and the analysis was carried out as described [22]. In brief, WGS (n = 4355) was analyzed using chewBBACA version 2.8.5 [23]. Firstly, a training file was created from the complete genome of *T. pallidum* (AE000520.1) using Prodigal and used in subsequent steps [24]. Secondly, a study-specific *T. pallidum* schema was created, and a FASTA file for each coding sequence (CDS) was generated. Thirdly, a BLAST database was created using the study-specific schema, herein referred to as the schema database. This was followed by a BLASTN search against the schema database using the *rpsC* and *rpsJ* genes from *T. pallidum* (AE000520.1) as queries. The multiple sequence alignment files were imported into CLC Genomics Workbench (v20), and the CDSs were translated. The presence of non-synonymous substitutions in the following proteins was further evaluated: rpsC (Lys4; His175) and rpsJ (Val57).

For 16S analysis, a BLASTN search using the 16S rRNA *T. pallidum* (AE000520.1) sequence was queried against the BLAST database that was created using all 4,355 genomes. A BLASTN search was also performed against *T. pallidum* isolate collection in PubMLST. Thus, for the 16S analysis, the dataset included the genomes and sequences from Genbank and PubMLST, respectively.

## Results

### *Mutations in the 16S rRNA gene*

 doi:10.20944/preprints202403.0563.v1



Our analyses revealed a transition mutation from TGA to TGG at positions 965 to 967 (*Escherichia coli* 16S rRNA numbering) observed among Treponema (5.6% occurrence) and Spirochaeta spp. (3.97% occurrence) genomes. Additional mutations at the same positions, including CGC, GGT, CGA, TGR(A/G), TGC and AGC, were detected less frequently (Table 1).

**Table 1.** List of the tetracycline resistance associated mutations.

| Ribosomal genes | Source of genomes | Organism | Total no of genomes | Mutations | No of genomes with mutations | No of genomes with 2 copies | No of genomes with >2 copies | Copy number |
|---|---|---|---|---|---|---|---|---|
| 16S rRNA | Genbank | *Treponema parvum* | 5 | TGA 965-967 TGG | 2 | 2 | | 1-2 copies |
| | | | | TGA 965-967 GGT | 1 | 0 | | 1 copy |
| | | *Treponema brennaborense* | 4 | TGA 965-967 TGG | 1 | 1 | | 4 copies |
| | | *Treponema peruense* | 4 | TGA 965-967 TGG | 1 | 1 | | 4 copies |
| | | *Treponema bryantii* | 5 | TGA 965-967 TGG | 2 | 0 | 3 | 1-4 copies |
| | | *Treponema sp.* | 60 | TGA 965-967 CGC | 1 | 0 | | 1 copy |
| | | Other *Treponema* spp. | 1341 | TGA 965-967 TGG | 71 | 24 | 9 | 1-3 copies |
| | | Spirochaeta spp. | 1006 | TGA 965-967 CGA | 8 | 0 | | 1 copy |
| | | | | TGA 965-967 TGG | 40 | 18 | | 1 to 5 copies |
| | PubMLST | *Treponema pallidum* | 544 | TGA 965-967 TGG | 1 | - | - | - |
| | | | | TGA 965-967 TGR(A/G) | 2 | - | - | - |
| | | | | TGA 965-967 TGC | 1 | - | - | - |
| | | | | TGA 965-967 AGC | 1 | - | - | - |
| rpsJ (30S ribosomal subunit protein S10) | Genbank | *Candidatus Borreliella tachyglossi* | 1 | V57A | 1 | - | - | - |
| | | *Treponema porcinum* | 2 | V57G | 2 | - | - | - |
| | | Treponema sp. | 60 | V57G | 12 | - | - | - |
| | | | | V57I | 1 | - | - | - |
| | | | | V57K | 3 | - | - | - |
| | | *Borrelia persica* | 1 | V57I | 1 | - | - | - |
| | | Spirochaeta spp. | 1006 | V57G | 1 | - | - | - |
| | | | | V57I | 4 | - | - | - |
| | | | | V57K | 17 | - | - | - |
| | | Other *Treponema* spp. | 1341 | V57K | 15 | - | - | - |
| rpsC (30S ribosomal subunit protein S3) | Genbank | Spirochaetales bacterium | 5 | H178Q | 4 | - | - | - |

- Not available or not determined.

 doi:10.20944/preprints202403.0563.v1



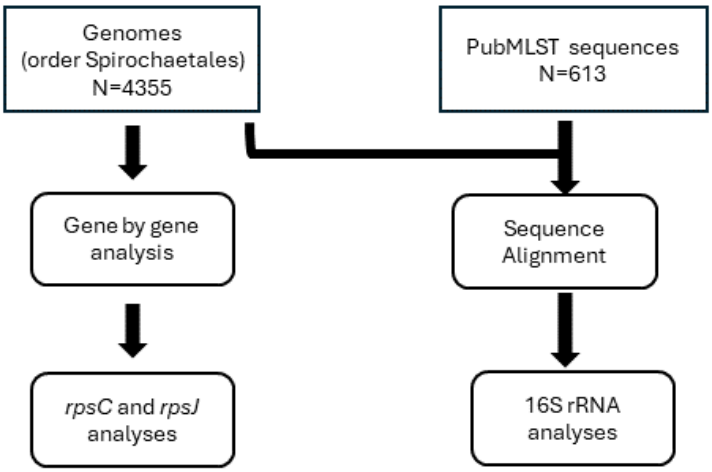


**Figure 1.** Flowchart describing genomes and sequences used in the analyses.

*Mutations in the rpsJ gene*

Analysis of the *rpsJ* gene, which encodes the 30S ribosomal subunit protein S10, showed the presence of the V57G substitution that was observed in both the *Treponema porcinum* genomes and 20% of *Treponema* sp. genomes (Table 1). The presence of additional variants, V57I and V57K, was also observed at a lower incidence (Table 1). The substitution V57K (169 GTG-AAG 171) frequently appeared in the Spirochaeta spp. (1.69% of genomes) and other Treponema spp. (1.12% of genomes) (Table 1).

*Mutations in the rpsC gene*

An H178Q substitution in the *rpsC* gene, associated with the 30S ribosomal subunit protein S3, was found in 80% of the genomes of the Spirochaetales bacterium (Table 1).

## Discussion

The emergence and dissemination of antimicrobial resistance (AMR) are of utmost concern in infectious disease management, particularly in the context of sexually transmitted infections (STIs) such as syphilis. The resurgence of syphilis incidence in various countries, including Belgium, necessitates innovative interventions to curb this trend [1]. One such intervention is the administration of doxycycline as post-exposure prophylaxis (doxy-PEP), which has demonstrated efficacy in reducing the incidence of syphilis and other STIs among men who have sex with men (MSM) [5]. However, the extensive use of doxycycline raises concerns about the potential induction of AMR, especially considering its critical role as an alternative to penicillin, the first-line treatment [10].

Our study focused on the potential chromosomal mutations associated with tetracycline resistance within the Spirochetales order. The genomic data from this order, including 4,355 whole genome sequences and an additional 613 16S rRNA sequences, were systematically analyzed for the presence of mutations in 16S rRNA and non-synonymous mutations in the *rpsC* and rpsJ genes, which encode the 30S ribosomal subunit proteins S3 and S10, respectively.

Our analysis revealed the transition mutation TGA to TGG at positions 965 to 967 (*Escherichia coli* 16S rRNA numbering) in the 16S rRNA gene of *Treponema* and *Spirochaeta* spp. genomes. The identification of this mutation across these species suggests a potential mechanism of resistance that might become more prevalent under selective pressure from increased doxycycline use [25]. Although less frequent, other mutations in this gene point to the potential for diverse resistance mechanisms within these genera.

In the *rpsJ* gene, the V57G amino acid substitution was present in both the genomes of *Treponema porcinum* and a significant subset of *Treponema* spp., and this mutation could confer a fitness advantage in the presence of tetracycline. The V57I and V57K substitutions were also observed but at lower frequencies. Notably, the GTG-AAG (V57K) mutation at nucleotide positions 169-171 was relatively common in Spirochaeta spp. and other *Treponema* spp.. The minimal inhibitory concentration (MIC) of *E.* coli to tetracycline with V57K substitution was found to be 0.75 μg/ml, and *Escherichia coli* with V57I substitution showed a slightly increased MICs to tigecycline and tetracycline [26]. Substitutions in V57 amino acid positions that confer resistance to tetracycline or tigecycline have been observed in both Gram-negative and Gram-positive bacteria, namely *E. coli, Neisseria gonorrhoeae, Neisseria meningitidis, Klebsiella pneumoniae, Bacillus subtilis*, *Enterococcus faecium, Enterococcus faecalis* and *Staphyloccus aureus* [26–29].

The *rpsC* gene analysis uncovered an H178Q (corresponding to the H175 position in *Streptococcus pneumoniae*) substitution in a substantial majority of the Spirochaetales bacterium genomes, highlighting the critical nature of this mutation within this specific bacterial group [30].

In light of the widespread use of doxy-PEP, the putative mutations associated with tetracycline resistance reported in this study provide a prerequisite for developing molecular methods for the rapid detection and surveillance of antibiotic-resistant *T. pallidum* in clinical specimens [31]. Our study contributes to the foundational knowledge necessary for guiding future studies and public health strategies to mitigate the spread of AMR in the context of STI management.

## References


1. Mitjà O, Padovese V, Folch C, et al (2023) Epidemiology and determinants of reemerging bacterial sexually transmitted infections (STIs) and emerging STIs in Europe. Lancet Reg Heal - Eur 34:100742. https://doi.org/https://doi.org/10.1016/j.lanepe.2023.100742
2. Molina J-M, Charreau I, Chidiac C, et al (2018) Post-exposure prophylaxis with doxycycline to prevent sexually transmitted infections in men who have sex with men: an open-label randomized substudy of the ANRS IPERGAY trial. Lancet Infect Dis 18:308–317. https://doi.org/10.1016/S1473-3099(17)30725-9
3. Luetkemeyer AF, Donnell D, Dombrowski JC, et al (2023) Postexposure Doxycycline to Prevent Bacterial Sexually Transmitted Infections. N Engl J Med 388:1296–1306. https://doi.org/10.1056/NEJMoa2211934
4. Molina JM, Bercot B, Assoumou L, et al (2023) ANRS 174 DOXYVAC: an open-label randomized trial to prevent STIs in MSM on PrEP. In: 30th Conference on Retroviruses and Opportunistic Infections (CROI). pp 2–22
5. Mårdh O, Plachouras D (2023) Using doxycycline for prophylaxis of bacterial sexually transmitted infections: considerations for the European Union and European Economic Area. Eurosurveillance 28:. https://doi.org/https://doi.org/10.2807/1560-7917.ES.2023.28.46.2300621
6. Vanbaelen T, Tsoumanis A, Kenyon C (2023) Total Antimicrobial Consumption in Doxycycline Postexposure Prophylaxis Cohorts and the Intensity of Screening for Bacterial Sexually Transmitted Infections. Clin Infect Dis ciad553. https://doi.org/10.1093/cid/ciad553
7. Vanbaelen T, Manoharan-Basil SS, Kenyon C (2023) Doxycycline Postexposure Prophylaxis Could Induce Cross-Resistance to Other Classes of Antimicrobials in Neisseria gonorrhoeae : An In Silico Analysis. Sex Transm Dis 50:490–493. https://doi.org/10.1097/OLQ.0000000000001810
8. Gestels Z, Manoharan-Basil SS, Kenyon C (2023) Doxycycline post exposure prophylaxis could select for cross-resistance to other antimicrobials in various pathogens: An in silico analysis. Int J STD AIDS 34:962–968. https://doi.org/10.1177/09564624231190108
9. Kenyon C (2024) Doxycycline post exposure prophylaxis could theoretically select for resistance to various antimicrobials in 19 pathobionts: an in silico analysis. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. https://doi.org/10.1016/j.ijid.2024.02.017
10. Nurse-Findlay S, Taylor MM, Savage M, et al (2017) Shortages of benzathine penicillin for prevention of mother-to-child transmission of syphilis: An evaluation from multi-country surveys and stakeholder interviews. PLoS Med 14:e1002473. https://doi.org/10.1371/journal.pmed.1002473
11. Wu B-R, Liu W-C, Wu P-Y, et al (2014) Surveillance study of Treponema pallidum harbouring tetracycline resistance mutations in patients with syphilis. Int. J. Antimicrob. Agents 44:370–372
12. Sanchez A, Mayslich C, Malet I, et al (2020) Surveillance of Antibiotic Resistance Genes in Treponema Pallidum Subspecies Pallidum from Patients with Early Syphilis in France. Acta Derm Venereol 100:adv00221. https://doi.org/10.2340/00015555-3589
13. Xiao H, Li Z, Li F, et al (2017) Preliminary study of tetracycline resistance genes in Treponema pallidum. J. Glob. Antimicrob. Resist. 9:1–2

14. Lukehart S. (2022) Could doxycycline PEP induce tetracycline resistance in Treponema pallidum?
15. Xiao Y, Liu S, Liu Z, et al (2016) Molecular Subtyping and Surveillance of Resistance Genes In Treponema pallidum DNA From Patients With Secondary and Latent Syphilis in Hunan, China. Sex Transm Dis 43:310–316. https://doi.org/10.1097/OLQ.0000000000000445
16. Beale MA, Marks M, Cole MJ, et al (2021) Global phylogeny of Treponema pallidum lineages reveals recent expansion and spread of contemporary syphilis. Nat Microbiol 6:1549–1560. https://doi.org/10.1038/s41564-021-01000-z
17. Mikalová L, Grillová L, Osbak K, et al (2017) Molecular Typing of Syphilis-Causing Strains Among Human Immunodeficiency Virus-Positive Patients in Antwerp, Belgium. Sex Transm Dis 44:376–379. https://doi.org/10.1097/OLQ.0000000000000600
18. Edmondson DG, Wormser GP, Norris SJ (2020) In Vitro Susceptibility of Treponema pallidum subsp. pallidum to Doxycycline. Antimicrob Agents Chemother 64:. https://doi.org/10.1128/AAC.00979-20
19. Hunfeld K-P, Kraiczy P, Kekoukh E, et al (2002) Standardized in vitro susceptibility testing of Borrelia burgdorferi against well-known and newly developed antimicrobial agents--possible implications for new therapeutic approaches to Lyme disease. Int J Med Microbiol 291 Suppl:125–137. https://doi.org/10.1016/s1438-4221(02)80024-8
20. Sicklinger M, Wienecke R, Neubert U (2003) In vitro susceptibility testing of four antibiotics against Borrelia burgdorferi: a comparison of results for the three genospecies Borrelia afzelii, Borrelia garinii, and Borrelia burgdorferi sensu stricto. J Clin Microbiol 41:1791–1793. https://doi.org/10.1128/JCM.41.4.1791-1793.2003
21. Moreno LZ, Miraglia F, Lilenbaum W, et al (2016) Profiling of Leptospira interrogans, L. santarosai, L. meyeri and L. borgpetersenii by SE-AFLP, PFGE and susceptibility testing--a continuous attempt at species and serovar differentiation. Emerg Microbes Infect 5:e17. https://doi.org/10.1038/emi.2016.16
22. Manoharan-Basil SS, Laumen JGE, Van Dijck C, et al (2021) Evidence of Horizontal Gene Transfer of 50S Ribosomal Genes rplB, rplD, and rplY in Neisseria gonorrhoeae . Front. Microbiol. 12:1263
23. Silva M, Machado MP, Silva DN, et al (2018) chewBBACA: A complete suite for gene-by-gene schema creation and strain identification. Microb genomics. https://doi.org/10.1099/mgen.0.000166
24. Hyatt D, Chen GL, LoCascio PF, et al (2010) Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. https://doi.org/10.1186/1471-2105-11-119
25. Pringle M, Fellström C, Johansson K-E (2007) Decreased susceptibility to doxycycline associated with a 16S rRNA gene mutation in Brachyspira hyodysenteriae. Vet Microbiol 123:245–248. https://doi.org/10.1016/j.vetmic.2007.02.019
26. Izghirean N, Waidacher C, Kittinger C, et al (2021) Effects of Ribosomal Protein S10 Flexible Loop Mutations on Tetracycline and Tigecycline Susceptibility of Escherichia coli. Front Microbiol 12:663835. https://doi.org/10.3389/fmicb.2021.663835
27. Williams G, Smith I (1979) Chromosomal mutations causing resistance to tetracycline in Bacillus subtilis. Mol Gen Genet 177:23–29. https://doi.org/10.1007/BF00267249
28. Villa L, Feudi C, Fortini D, et al (2014) Genomics of KPC-producing Klebsiella pneumoniae sequence type 512 clone highlights the role of RamR and ribosomal S10 protein mutations in conferring tigecycline resistance. Antimicrob Agents Chemother 58:1707–1712. https://doi.org/10.1128/AAC.01803-13
29. Cattoir V, Isnard C, Cosquer T, et al (2015) Genomic analysis of reduced susceptibility to tigecycline in Enterococcus faecium. Antimicrob Agents Chemother 59:239–244. https://doi.org/10.1128/AAC.04174-14
30. Lupien A, Gingras H, Leprohon P, Ouellette M (2015) Induced tigecycline resistance in Streptococcus pneumoniae mutants reveals mutations in ribosomal proteins and rRNA. J Antimicrob Chemother 70:2973–2980. https://doi.org/10.1093/jac/dkv211
31. Stamm L V (2010) Global challenge of antibiotic-resistant Treponema pallidum. Antimicrob Agents Chemother 54:583–589. https://doi.org/10.1128/AAC.01095-09