

Use of the MLST Technique to Describe the Allelic Profile of *Treponema Pallidum* in CSF Samples: A Systematic Review

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Submitted: 17 Jun 2022; Accepted: 23 Jun 2022; Published: 28 Jun 2022.

Citation: Isabelle de Carvalho Rangel, Beatriz Pereira de Azevedo, Marcia Quinhones Pires Lopes, Mariana Munhoz Rodrigues, Ricardo de Souza Carvalho, Fernando Raphael de Almeida Ferry. (2022). Use of the MLST Technique to Describe the Allelic Profile of *Treponema Pallidum* in CSF Samples: A Systematic Review. *Archives of Infect Diseases & Therapy*, 6(2),169-173.

Abstract

Syphilis is a sexually transmitted infection that has recently become a serious public health problem in Brazil. At the same time, the World Health Organization estimates that among 56 million individuals are infected with syphilis. Multilocus Sequence Typing consists of a high resolution molecular typing technique that is based on the amplification and sequencing of fragments of conserved genes called housekeeping (loci). The technique was recently developed and used for the study of syphilis, but there are few studies related to neurosyphilis (NS), a nosological entity with greater severity and morbidity. For *Tp* typing, three genes are identified: TP0136, TP0548 and TP0705. This systematic review aimed to analyze original studies that addressed the MLST technique, describing the allelic profile and discrimination of *Tp* clades present in each population and its relationship with clinical-epidemiological aspects, especially in neurosyphilis, through a bibliographic survey in databases with the following DECS: MLST, Multilocus Sequence Typing and *Treponema pallidum*. As a result, 56 articles were found, of which only 7 were selected for full reading. After reading the selected articles, only two reported CSF analysis. It is concluded that the determination of the allelic profile through MLST is a high-resolution genotyping tool that allows the distinction of subspecies and species of *Treponema pallidum*, and can be used for differentiation of strains within each phylogenetic tree of these microorganisms. The mutation most associated with macrolide resistance is A2058G, which is commonly found in Clade SS14-like samples, in addition, the most studied gene to assess macrolide resistance is 23s rRNA. The MLST technique presents a great perspective for the deepening of questions related to the study of different strains of *Tp*, especially the characterization of neurotropic strains that cause NS, antibiotic resistance, phylogeny and geographic distribution.

Keywords: MLST, Multilocus Sequence Typing and *Treponema Pallidum*

Introduction

Syphilis is a sexually transmitted infection that has recently become a serious public health problem in Brazil (PCDT). The World Health Organization (WHO) estimates that among 18 million organizations are infected with syphilis [1, 2]. *Treponema pallidum* subsp. *pallidum* (*Tp*) the causative agent of syphilis was first identified by Schaudinn and Hoffman in 1905, since

then it has spread and is diagnosed globally [3, 4].

The disease manifests itself in three different stages:

- Primary:** Syphilis that progresses with the appearance of painless sores on genital organs with a hardened base (hard chancre) in addition to lymphadenomegaly, which disappear spontaneously and do not leave scars

- b. **Secondary:** In this reddish spots appear on the skin, mouth mucosa, palms and soles of the feet; may be accompanied by fever; headache; malaise; in appetite; generalized lymphadenopathy, these manifestations may regress without treatment, although the disease remains active, resulting in tertiary syphilis
- c. **Tertiary:** There is a long asymptomatic or oligo symptomatic period, in which the bacterium remains latent, symptoms, may appear after years and with aggressiveness resulting in complications eye diseases and blindness, skin disease, osteopathies, cardiovascular manifestations and central nervous system disease leading to important neurological and mental disorders.

Another major public health problem is congenital syphilis resulting in fetal malformation, miscarriage and fetal death. Pneumonia, body wounds, osteo-articular disorders, blindness and mental development disorders appear in the first months of life. Since 1905, several techniques have been developed and used for the diagnosis and study of *Tp*; however, more recently the characterization of the genetic profile has been used with relative frequency. The determination of the *Tp* allelic profile, through the Multilocus Sequence Typing (MLST) technique, allows the distinction of its subspecies and species, and can be used to differentiate strains within each of these phylogenetic branches of microorganisms [5].

Multilocus Sequence Typing consists of a high-resolution molecular typing technique. It is based on the amplification and sequencing of conserved gene fragments called housekeeping (loci). In the case of *Tp*, three genes are identified: TP0136, TP0548 and TP0705. When combined, these genes correspond to about 30% of its complete genome [5]. Each isolate is identified according to the allelic profile generated from the sequences of each analyzed gene. This allele profile is called Sequence Type (ST), which corresponds to a randomly generated numerical sequence [6, 7].

MLST is an excellent tool to assess diverse information about syphilis in clinical and epidemiological aspects, antibiotic resistance, genetic profiling, phylogenetic and evolutionary selection in different geographic areas [8-10]. MLST has been used in hundreds of clinical samples - from patients diagnosed with syphilis - in several clinical and epidemiological studies around the world, such as: Czech Republic, Switzerland, France, Cuba, China and Spain; in these countries, it was found that the presence of the A2058G mutation in the 23s RNA gene, found in samples of the SS14 clade, is commonly associated with macrolide resistance, making the treatment of this disease difficult [5,8,9,11-13].

The MLST technique was recently developed and used for the study of syphilis, but there are few studies related to neurosyphilis (NS), a nosological entity with greater severity and morbidity. Sahi and his collaborators (2021) through the MLST technique found and described subtypes with greater neurotropic potential.

Thus, MLST may present an enormous potential for studying this condition. NS occurs by the invasion of *Tp* in the Central Nervous System (CNS), in any period of syphilis, and may or may not cause manifestation of neurological symptoms. The laboratory diagnosis of NS can be performed by the positivity of the *Veneral Disease Research Laboratory* in the cerebrospinal fluid (VDRL-CSF), or, in its absence, by the combination of treponemal tests in the cerebrospinal fluid (CSF), in addition to the increase in cellularity and levels of CSF proteins [14].

One of the advantages of using the MLST technique is that it allows distinguishing the allelic profile based on several nucleotide differences, which allows the distinction of *Tp* from other treponemal species and subspecies [15]. However, it is an expensive technique, requiring a laboratory structure, specialized technical training in addition to high-cost inputs and the need for constant updating of professionals. This systematic review aimed to analyze original studies that addressed the MLST technique, describing the allelic profile and discrimination of *Tp* clades present in each population and its relationship with clinical-epidemiological aspects, especially in neurosyphilis.

Materials and Methods

For this survey, an electronic search was carried out in the following databases: EMBASE, Scielo, MEDLINE/PUBMED, LILACS, Cochrane Library with the descriptors in English, Portuguese and Spanish: “*Treponema pallidum*” and “MLST” or “*Multilocus Sequence Typing*”. The same descriptors were also searched in Trials databases (Clinical Trials and ICTRP) as well as in the Brazilian bases of "gray literature" in the Theses and Dissertations Catalog (CAPES) and in the Brazilian Digital Library of Theses and Dissertations. The search was limited to the period from January 2018 to January 2022, in order to select mainly recent studies on the use of molecular *Tp* genotyping, neurotropic potential and geographic distribution of subfamilies (Clado).

Inclusion criteria

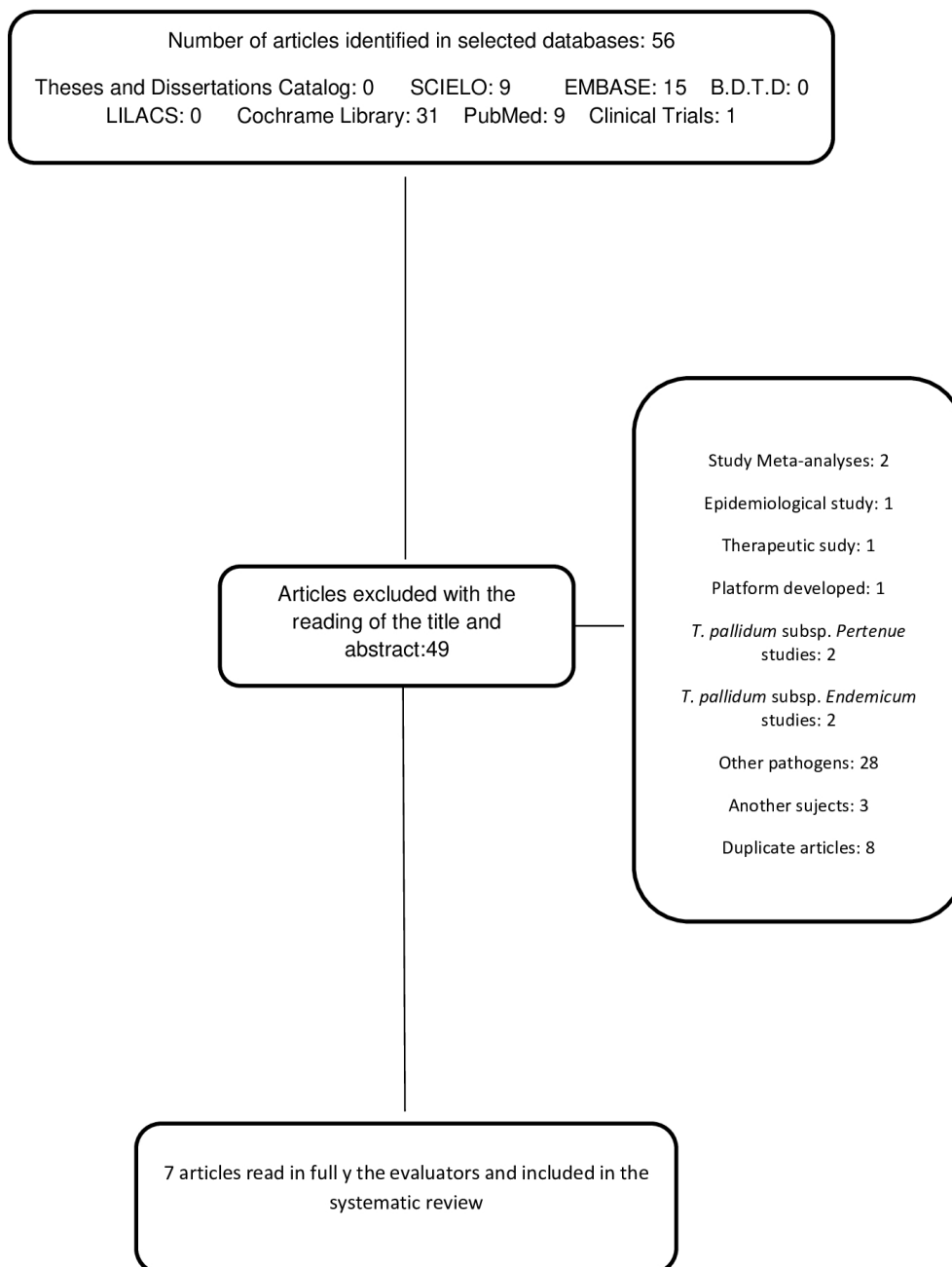
The following inclusion criteria were used: studies involving molecular typing of *Treponema pallidum* subsp. *pallidum* by the MLST technique.

Exclusion Criteria

The following were excluded: meta-analyses, epidemiological, therapeutic studies that addressed other *Tp* subspecies, other pathogens or that addressed other issues that were not related to syphilis.

Results/Discussion

Fifty-six articles were identified in the following databases: 0 in Scielo, 9 in Pubmed, 0 in LILACS, 1 in Clinical Trials, 15 in Embase, 31 in the Cochrane Library and 0 in the CAPES Theses and Dissertations Catalog. Of these 56 articles, 7 were selected for full reading, and 49 were excluded after reading the title and abstract according to Flowchart 1.



Flowchart 1: Flow of sorting the articles found in the databases

The main reasons for excluding articles were: 2 meta-analysis studies, 1 epidemiological study, 1 therapeutic study, 1 described the development of the PubMLST platform, 4 articles talked about *Treponema pallidum* subsp. *perternue*, 1 article addressed *Treponema pallidum* subsp. *endemicum*, 3 articles addressed other issues unrelated to syphilis, 8 duplicate articles and 28 addressed other pathogens.

It was observed that the 7 selected articles included a total of 574 patients and 1,169 biological samples that corresponded to the inclusion criteria proposed in this review. Of these 1,169 samples, 199 of blood, 522 of swab, 4 of tissue and 50 of CSF and 2 articles do not describe the types of samples analyzed. Most of the bacteria that cause diseases are isolated through laboratory cultivation but only in 2018 Edmondson et al were able to multiply and maintain *Tp* in the laboratory through a tissue culture

system, preserving its morphological and infectious properties for a long period, effectively, but this promising technique is not yet available for most research and diagnostic centers. Further refinement of this long-term subculture system is expected to facilitate the study of the physiological, genetic, pathological, immunological and antimicrobial susceptibility properties of *T. pallidum* subsp. *pallidum* and closely related pathogenic species and subspecies of *Treponema*.

Molecular biology has shown to be effective for the detection and genotyping of *Tp*, the polymerase chain reaction has high sensitivity and specificity, as well as the most modern techniques of genetic sequencing such as MLST [5, 16]. Studies related to the use of MLST may bring important clinical and epidemiological considerations. Six of the seven articles included in this review reported that the presence of the A2058G or A2059G

mutation in 23S rRNA, found in samples from the SS14 clade is commonly associated with macrolide resistance [17, 18]. In China, mutations associated with macrolide resistance probably occurred as a result of the indiscriminate and excessive use of this class of antibiotics to treat respiratory tract infections and sexually transmitted infections and not by dissemination arising from mutant strains from just a few individuals, this conclusion is based on the fact that macrolides are rarely prescribed for the treatment of syphilis [9, 19].

Of the 7 articles included in the study, only two performed CSF analysis. The results describe that patients with the 1.1.2 allelic profile, discriminated by the MLST technique, correspond to the 14d/f subtype described by the Enhanced Centers for Disease Control technique in Prevention Typing (ECDCT), thus, the 1.1.2 alleles (14d/ f) were more likely to develop neurosyphilis. Unlike patients with allelic profile 1.3.1 (14 d/g), they had a lower propensity to develop the disease. In addition, the authors report that the two main genes of clinical relevance related to neurosyphilis are Tp0548 type f and Tp0705 type, 2 as patients with clinical/laboratory reports compatible with neurosyphilis had these genes in their clinical sample. In clinical practice, if these two genes were detected from the laboratory analysis of the patient's blood sample, the patient would benefit from not needing a lumbar puncture [8].

Liu et al studying the Chinese population of Xiamen, observed that the allelic profile most commonly found in biological samples containing Tp was 1.1.8, but did not relate it to NS, despite using CSF in their study [8]. He also mentions that in other countries, the most common allelic profile is 1.1.3, suggesting that the strains tend to remain restricted to certain geographic areas, as it has not yet been introduced in China. The article published by Liu et al is the only one that addresses the use of CSF samples for Tp genotyping using the MLST technique. In this way, we have an excellent tool and great potential for the study of neurosyphilis, which will allow the detection of potentially neurotropic strains, which present greater severity, morbidity and harm to patients [8].

Conclusion

It is concluded that the determination of the allelic profile through MLST is a high-resolution genotyping tool that allows the distinction of subspecies and species of *Treponema pallidum*, and can be used to differentiate strains within each phylogenetic tree of these microorganisms. In Brazil, no studies related to Tp genotyping were found. The mutation most associated with macrolide resistance is A2058G, which is commonly found in Clade SS14-like samples, in addition, the most studied gene to assess macrolide resistance is 23s rRNA. The MLST technique presents a great perspective for the deepening of questions related to the study of different strains of Tp, especially the characterization of neurotropic strains that cause NS, antibiotic resistance, phylogeny and geographic distribution.

List of abbreviations

PCDT: Clinical protocols and therapeutic guidelines
Tp: *Treponema Pallidum* Subsp. *Pallidum*
MLST: Multilocus Sequence Typing

loci: Housekeeping
ST: Sequence Typing
NS: Neurosyphilis
CNS: Central Nervous System
CSF-VDRL: Venereal Disease Research Laboratory in the Cerebrospinal Fluid
CAPES: Theses and Dissertations Catalog
CSF: Cerebrospinal Fluid
ECDCT: Enhanced Centers for Disease Control and Prevention Typing

Author Contributions

All authors contributed to the conception and design of the study. Beatriz Azevedo and Isabelle Rangel performed material preparation, data collection and analysis. Isabelle Rangel wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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