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Diagnosis, molecular typing, and resistance profile of *Treponema pallidum*

Diagnóstico, tipagem molecular e perfil de resistência do *Treponema pallidum*

Diagnóstico, tipificación molecular y perfil de resistencia de *Treponema pallidum*

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ABSTRACT

Objective: To analyze the epidemiological and molecular characteristics of syphilis and discuss molecular approaches for optimizing the early diagnosis of *Treponema pallidum* infection. **Literature review:** The incidence of syphilis, a sexually transmitted infection (STI), has been increasing worldwide, with an estimated 8.0 million new cases reported in 2022. Currently, syphilis is diagnosed using serological tests, which are associated with limitations, such as low sensitivity to detect early-stage infection and poor ability to distinguish between cured and active syphilis cases. Thus, there is an urgent need to develop new diagnostic methods to detect *Treponema pallidum* in the early stages of infection. Molecular typing methods, including the Enhanced Centers for Disease Control and Prevention system, help in understanding the global diversity of *T. pallidum* strains. Despite its relevance, there is a scarcity of studies on the molecular profile and resistance of *T. pallidum* in South America. **Final considerations:** It is essential that public health efforts focus not only on education and awareness of prevention but also on research and development of new diagnostic and therapeutic strategies.

Keywords: Syphilis, Public health, European Centre for Disease Prevention and Control (ECDC).

RESUMO

Objetivo: Analisar os fundamentos e a epidemiologia da sífilis, assim como discutir as abordagens moleculares que podem ser utilizadas para otimizar o diagnóstico precoce da infecção. **Revisão bibliográfica:** A incidência de sífilis tem aumentado globalmente, com cerca de 8,0 milhões de novos casos estimados em 2022, gerando preocupação em relação a essa infecção sexualmente transmissível (IST). Embora o diagnóstico atual é realizado com testes sorológicos, eles apresentam limitações, como baixa sensibilidade no início da infecção e dificuldade em diferenciar entre sífilis tratada e ativa. Isso ressalta a necessidade de desenvolvimento de novos métodos diagnósticos, que permitam a detecção de *T. pallidum* em estágios iniciais da infecção. Por outro lado, a tipagem molecular, incluindo o sistema Enhanced Centers for Disease Control and Prevention (ECDC), ajuda a entender a diversidade das cepas de *T. pallidum* globalmente. Apesar da relevância, há escassez de estudos sobre o perfil molecular e resistência do *T. pallidum* na América do Sul. Descrever todos os pontos metodológicos de forma sucinta, público, localização, coleta de dados e instrumento de pesquisa. **Considerações finais:** É fundamental que os esforços em saúde pública se concentrem não apenas na educação e conscientização sobre prevenção, mas também na pesquisa e no desenvolvimento de novas estratégias diagnósticas e terapêuticas.

Palavras-chave: Sífilis, Saúde pública, Centro Europeu de Prevenção e Controle de Doenças (ECDC).

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RESUMEN

Objective: Analizar los fundamentos y la epidemiología de la sífilis, así como discutir los enfoques moleculares que pueden utilizarse para optimizar el diagnóstico precoz de la infección. **Revisión Bibliográfica:** La incidencia de sífilis ha aumentado a nivel global, con aproximadamente 8,0 millones de nuevos casos estimados en 2022, lo que genera preocupación respecto a esta infección de transmisión sexual (ITS). Aunque el diagnóstico actual se realiza mediante pruebas serológicas, estas presentan limitaciones, como baja sensibilidad al inicio de la infección y dificultad para diferenciar entre sífilis tratada y activa. Esto resalta la necesidad de desarrollar nuevos métodos de diagnóstico que permitan la detección de *T*. *pallidum* en las etapas tempranas de la infección. Por otro lado, la tipificación molecular, incluido el sistema Enhanced Centers for Disease Control and Prevention (ECDC), ayuda a comprender la diversidad de las cepas de *T*. *pallidum* a nivel global. A pesar de su relevancia, existe una escasez de estudios sobre el perfil molecular y la resistencia de *T*. *pallidum* en América del Sur. Todos los puntos metodológicos, como público, ubicación, recolección de datos e instrumentos de investigación, deben describirse de forma concisa. **Consideraciones finales:** Es fundamental que los esfuerzos de salud pública se centren no solo en la educación y concienciación sobre la prevención, sino también en la investigación y desarrollo de nuevas estrategias diagnósticas y terapéuticas.

Palabras clave: Sífilis, Salud pública, Centro Europeo para la Prevención y el Control de las Enfermedades (ECDC).

INTRODUCTION

Globally, the incidence of syphilis, a sexually transmitted infection (STI), has been increasing, with an estimated 8.0 million new cases reported in 2022 (WHO, 2024). In Brazil, the number of cases of acquired syphilis in pregnant women and congenital syphilis markedly increased between 2012 and 2022. The detection rate of acquired syphilis increased from 14.1 to 99.2 per 100,000 inhabitants, while that of syphilis in pregnant women increased from 5.7 to 32.4 per 1,000 live births. Additionally, the detection rate of congenital syphilis increased from 4 to 10.3 per 1,000 live births (BRASIL, 2023).

Syphilis diagnosis is performed through serological tests, clinical manifestations, and patient history. However, serological tests have some limitations, such as low sensitivity at the onset of infection and an inability to distinguish between treated syphilis cases and active infection (PEELING RW, et al., 2023). These limitations highlight the importance of developing new diagnostic methods. The detection of *T. pallidum* through Polymerase Chain Reaction (PCR) has been described in some clinical samples and stages of syphilis, including early infection lesions where serological tests are not yet reactive (QUEIROZ JHFS, et al., 2024). Additionally, molecular typing has helped in understanding the transmission of the infection, with the Enhanced Centers for Disease Control and Prevention (ECDC) typing system being used to identify the diversity of *T. pallidum* isolates in different countries (MARRA CM, et al., 2010).

The first molecular typing method for *T. pallidum* was developed by evaluating the variations in the acidic repeat protein (arp)-encoding gene and the *tpr* (*T*. *pallidum* repeat) genes of subfamily II (tprE [tp0313], tprG [tp0317], and *tpr*J [tp0621]) (PILLAY A, et al.,1998). In 2010, this typing method was improved with the inclusion of a new molecular marker (the treponemal *tpr*0548 gene). This novel typing technique, known as ECDC, is based on the analysis of the three specific regions of *T. pallidum* (*arp*, *tpr*EGJ, and tpr0548 genes) (MARRA CM, et al., 2010).

There is a lack of studies on the molecular profile and resistance of *T. pallidum* in South America. Thus, this review aims to address general and epidemiological aspects of syphilis and to contextualize molecular methods that can be used to enhance early disease diagnosis and support the understanding of infection transmission. These methods also enable the characterization of isolates, monitoring of treatment efficacy, and analysis of mutations associated with antibiotic resistance.

LITERATURE REVIEW

Syphilis

Syphilis is a treatable, multi-system infectious disease caused by *T. pallidum*, an invasive, obligate extracellular spirochete (TIECCO G, et al., 2021). The transmission of *T. pallidum* occurs through sexual contact with an infected partner. The spirochetes penetrate the mucous membranes or skin of the patient. To establish the infection, *T. pallidum* must adhere to epithelial cells and the extracellular matrix. Additionally, *T. pallidum* can infiltrate lymph nodes and spread in the host (PEELING RW, et al., 2023). Syphilis is clinically divided into different stages or phases. In the primary stage, the main clinical manifestation is the appearance of one or more chancres in the genital or extragenital area of the patient approximately 10 to 90 days after infectious contact (mainly sexual transmission). Primary chancres are typically painless, which may delay patients from seeking medical care. Primary phase symptoms can disappear without treatment in approximately 45 days, potentially leading to a false perception of cure by the patient (TUDOR ME, et al., 2023).

After the primary stage, some patients may progress to the secondary stage. This stage is characterized by multiple lesions in different body parts, including the mouth, genital, and anal areas, palms of the hands, and soles of the feet. The secondary stage is considered the most infectious stage of syphilis (TUDOR ME, et al., 2023). Without treatment, the clinical manifestations of the secondary stage disappear in approximately 45 days. The patient then remains asymptomatic for years. This stage can be termed the early latent stage, when the asymptomatic period lasts up to a year, or the late latent stage, when the asymptomatic period lasts for more than a year (PEELING RW, et al., 2023). If untreated, *T. pallidum* can evade the host immune system and affect vital organs, such as the heart and central nervous system (PEELING RW, et al., 2023). Early diagnosis and appropriate treatment are crucial for preventing these complications.

Epidemiology of Syphilis

In the last two decades, syphilis cases have tripled worldwide, with an annual number of new cases estimated to be 6.3 million. According to the World Health Organization, the number of new syphilis cases among adults aged 15 to 49 years increased from 7.1 million in 2020 to 8.0 million in 2022.

In 2022, 390,000 adverse birth outcomes related to maternal syphilis infection were reported, including 150,000 early fetal deaths and stillbirths, 70,000 neonatal deaths, 55,000 preterm or low-weight births, and 115,000 infants diagnosed with congenital syphilis. Thus, there is an urgent need to improve syphilis prevention and ensure access to testing and treatment (WHO, 2024).

Although syphilis is common among individuals from disadvantaged socioeconomic backgrounds and in vulnerable populations, its incidence has significantly increased in high-income countries, including the United States, Canada, Australia, Japan, and several European countries. In the United States, the Centers for Disease Control reported 51.5 cases per 100,000 people, with over 2.5 million new syphilis cases recorded in 2022 (CHAUDHRY S, et al., 2023; ECDC, 2024).

According to the European Center for Disease Prevention and Control, 35,391 syphilis cases were reported in 29 European Union countries in 2022, reflecting a 34% increase from 2021 and a 41% increase from 2018. Syphilis rates in men were eight times higher than those in women (ECDC, 2024). In these countries, the most affected groups include men who have sex with men and heterosexual women. The increased incidence of syphilis is often linked to behavioral factors (such as a high number of sexual partners). Additionally, the effective implementation of human immunodeficiency virus (HIV) treatments and pre-exposure prophylaxis resulted in decreased condom usage, contributing to increased incidences of syphilis (CHAUDHRY S, et al., 2023).

Data from the Ministry of Health revealed that acquired syphilis cases in Brazil increased by 662% between 2012 and 2022 (from 27,964 in 2012 to 213,129 in 2022). From 2012 to 2022, 1,237,027 acquired syphilis cases, 537,401 cases of syphilis in pregnant women, 238,387 cases of congenital syphilis, and 2,153 congenital syphilis deaths have been reported. In Brazil, syphilis case reporting is mandatory for all

health services. The data are collected through the Notifiable Diseases Information System (SINAN). Mato Grosso do Sul recorded the second-highest acquired syphilis detection rate in the Midwest region in 2022 (104 cases per 100,000 inhabitants) (BRAZIL, 2023).

The prevalence of syphilis in Brazil varies depending on the region and socioeconomic status. The syphilis prevalence rates in patients with HIV infection, patients with tuberculosis, and pregnant women are 41.81%, 17.27%, and 10%, respectively (QUEIROZ JHFS, et al., 2024). The number of syphilis cases has increased among the indigenous people both in Brazil and other parts of the world. For example, the number of syphilis cases among indigenous people increased from 290 in 2015 to 901 in 2022, which represents a 300% increase (BRAZIL, 2023). These data indicate the urgent need for developing effective strategies for syphilis prevention, diagnosis, and treatment to curb the rising incidence of this infection across various populations worldwide.

Syphilis Diagnosis

Patients with suspected primary or secondary syphilis presenting with lesions are diagnosed by identifying *T. pallidum* in the lesions. Direct diagnostic techniques for detecting *T. pallidum* have evolved from dark-field microscopy (observing pathogen motility) to tests involving silver staining or fluorescent anti-treponema antibodies. Recently, molecular techniques have been developed to detect *T. pallidum* (PEELING RW, et al., 2023). These methods were developed to address the decreased sensitivity of serological tests for primary-stage syphilis and the challenges in interpreting serological test results for diagnosis and treatment monitoring (TIECCO G, et al., 2021).

Serological tests are an indirect method for laboratory diagnosis of syphilis and are classified into the following two types: treponemal tests (identification of *T. pallidum*-specific antibodies) and non-treponemal serological tests (detection of non-specific anticardiolipin antibodies that are released during infection by host cells and the pathogen) (TIECCO G, et al., 2021).

The efficacy of syphilis treatment can be monitored based on the gradual decrease in anti-lipid antibody titers in the blood of patients. In patients with syphilis, the titers of antibodies in the Venereal Disease Research Laboratory test gradually decrease when the treatment is effective. However, the anti-*T. pallidum* antibodies may remain in circulation for months to years after cure (TUDDENHAM S, et al., 2020). WANG C, et al. (2021) followed nine patients with syphilis (primary or secondary) and quantified treponemal DNA in their saliva samples post-treatment. *T. pallidum* DNA was undetectable at approximately 64 h post-treatment (WANG C, et al., 2021). However, few studies address treatment follow-up using molecular techniques across all stages of syphilis.

T. pallidum DNA has been identified in saliva and plasma samples from patients at all stages of syphilis. For this, Nested-PCR for *pol*A and *tpp*47 genes was used. In clinical saliva samples, researchers found high levels of *T. pallidum* DNA, suggesting that this secretion may be an alternative route of syphilis transmission. Additionally, saliva collection is advantageous for *T. pallidum* detection for diagnosis due to its less invasive nature (WANG C, et al., 2021).

Molecular Characterization of *Treponema pallidum*

The *T. pallidum* genome is conserved and contains polymorphic regions, which can be used for molecular typing of the pathogen. Recently, molecular typing methods have been developed for characterizing *T. pallidum*. Molecular typing enables the epidemiological studies of *T. pallidum*, monitoring of its geographic distribution, and the differentiation between treponemas associated with specific clinical symptoms, such as neuroinvasive *T. pallidum* strains (MARRA CM, et al., 2010).

The first molecular typing method for *T. pallidum* was developed by PILLAY A, et al. (1998) and named the Centers for Disease Control and Prevention (CDC) typing system. This technique detected the number of 60-base-pair tandem repeats occurring in the acidic repeat protein gene (*arp*) (*tpr*0433) and analyzed restriction fragment length polymorphism (RFLP) of the *T. pallidum repeat* family genes (*tpr*E [tp0313], *tpr*G [tp0317], and *tpr*J [tp0621]) (PILLAY A, et al., 1998).

In 2010, the CDC molecular typing technique was enhanced to include analysis of a region of the tp0548 gene and was named the Enhanced CDC (ECDC) typing (MARRA CM, et al., 2010). The ECDC typing technique was tested in 25 studies across 18 countries using 3,014 clinical samples. Of these, complete typing was achieved in 69.87% (2,106/3,014) of samples, identifying 167 subtypes (DAI T, et al., 2012; FERNÁNDEZ-NAVAL C, et al., 2019; FLORES JA, et al., 2016; GIACANI L, et al., 2018; GRANGE PA, et al., 2013; GRIMES M, et al., 2012; KANAI M, et al., 2019; KHAIRULLIN R, et al., 2016; KOJIMA Y, et al., 2019; KUBANOV AA, et al., 2017; LIU D, et al., 2021; LU Y, et al., 2017; MIKALOVÁ L, et al., 2013; MIKALOVÁ L, et al., 2017; PENG R, et al., 2012; READ P, et al., 2016; SALADO-RASMUSSEN K, et al., 2016; SATO NS, et al., 2017; SHUEL M, et al., 2018; VAULET LG, et al., 2017; VENTER JME, et al., 2021; WU H, et al., 2012; XIAO Y. et al., 2016; YANG CJ, et al., 2015; ZONDAG HCA, et al., 2020). **Table 1** describes the 19 most prevalent subtypes, representing 82% of *T. pallidum* isolates identified using the ECDC method.

Molecular typing is efficient for cultured strains or clinical lesion samples due to the high concentration of specific bacterial DNA that can be isolated without interference from other nucleic acids, especially human DNA. Despite recent advances, *in vitro T. pallidum* culture from patient clinical samples remains a challenge and is rarely performed (EDMONDSON DG, et al., 2018).

Table 1- The 19 most prevalent *T. pallidum* subtypes using the ECDC method from 2106 isolates across 18 countries.
*Additional 148 *T. pallidum* subtypes using the ECDC method.

	Subtypes ECDC																						
Country	6d/f	11d/f	13d/f	14a/f	14b/f	14b/g	14d/d	14d/f	14d/g	14e/f	14e/g	14f/f	14f/g	14j/g	14k/g	14l/g	14p/g	15d/f	16d/f	Outros*	Period	References
USA	-	-	-	-	-	-	-	42	57	-	-	-	-	-	-	-	-	11	-	13	2004-2011	GRIMES M, et al. (2012)
Canada	-	-	-	-	-	2	-	1	36	-	-	-	4	3	2	1	4	-	-	4	2012-2016	SHUEL M, et al. (2018)
Brazil	-	-	-	-	-	-	5	-	6	-	-	-	-	-	-	-	-	-	-	1	2013-2015	SATO NS, et al. (2017)
Argentina	-	2	-	-	-	-	6	6	2	-	-	-	-	-	-	-	-	-	-	5	2006-2013	VAULET LG, et al. (2017)
Peru	-	-	-	-	-	-	-	3	2	-	-	-	-	-	-	-	-	-	-	2	2013-2014	FLORES JA, et al. (2016)
South Africa	-	1	3	-	2	-	-	26	5	5	-	-	-	-	-	-	-	5	1	47	2010-2018	VENTER JME, et al. (2021)
Denmark	-	-	-	-	3	7	1	35	107	-	4	3	7	3	2	10	3	-	-	12	2009-2013	SALADO-RASMUSSEN K, et al. (2016)
France	-	-	-	-	-	-	1	19	49	-	-	-	-	-	-	-	-	1	-	1	2005-2012	GRANGE PA, et al. (2013)
Czech Republic	-	-	-	-	-	-	-	9	4	1	1	-	-	-	-	-	-	2	-	1	2006-2012	MIKALOVÁ L, et al. (2013)
Belgium	-	-	-	-	-	-	-	-	1	2	-	-	-	2	-	-	1	-	-	3	2014-2015	MIKALOVÁ L, et al. (2017)

Spain	-	-	-	1	-	5	-	5	17	-	1	3	9	1	1	1	4	-	-	14	2015	FERNÁNDEZ-NAVAL C, et al. (2019)
Italy	1	-	-	2	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	17	2016-2017	GIACANI L, et al. (2018)
Netherlands	-	-	-	-	1	-	5	9	23	3	3	-	1	-	11	-	-	-	-	44	2016-2017	ZONDAG HCA, et al. (2020)
Australia	-	3	-	-	2	11	2	18	92	1	7	-	2	7	6	6	6	2	-	26	2004-2011	READ P, et al. (2016)
Japan	-	1	2	-	-	-	-	63	3	1	-	1	-	-	-	-	-	-	-	16	2013-2017	KOJIMA Y, et al. (2019); KANAI M, et al. (2019)
China	16	10	31	25	3	-	-	438	2	13	-	-	-	-	-	-	-	43	36	136	2007-2017	PENG R, et al. (2012); DAI T, et al. (2012); XIÃO Y. et al. (2016); LU Y, et al. (2017); LIU D, et al. (2021)
Taiwan	-	-	-	5	2	-	-	-	-	2	-	46	-	-	-	-	-	-	-	26	2009-2011	WU H, et al. (2012); YANG CJ, et al. (2015)
Russia	-	-	-	-	3	-	-	164	2	-	-	-	-	-	-	-	-	-	-	10	2013-2016	KHAIRULLIN R, et al. (2016); KUBANOV AA, et al. (2017)
Total	**17**	**17**	**38**	**31**	**16**	**25**	**20**	**838**	**429**	**28**	**16**	**53**	**23**	**16**	**22**	**18**	**18**	**66**	**37**	**378**	**2004-2018**	**25 studies**

Source: Authors.

Multilocus sequence typing (MLST), a molecular typing method for *T. pallidum* isolates, enables *T. pallidum* identification and subspecies differentiation. In the MLST technique, the sequences of the tp0136, tp0548, and tp0705 genes are analyzed. To assess the stability of these genes as a typing method, 11 serial passages of the *T. pallidum* reference strain in rabbits were analyzed. All passages exhibited the same subtype, indicating that the stability in these typing genes was equivalent to more than a hundred treponemal generations (GRILLOVA L, et al., 2019).

A meta-analysis of 43 studies revealed that the overall efficiency of *T. pallidum* typing methods was 71.4% (95% confidence interval (CI): 63.2%–78.9%). Subgroup analysis demonstrated that the efficiencies of the ECDC and MLST methods were 72.3% (95% CI: 60%–83.1%) and 67.1% (95% CI: 61.1–72.7%), respectively (FU B, et al., 2020). Comparative analysis of the ECDC and MLST techniques demonstrated that neurosyphilis was frequent in individuals infected with *T. pallidum* harboring the type f tp0548 gene (ECDC) or the type 2 tp0705 gene (MLST). This indicates the potential clinical relevance for identifying patients with neurosyphilis. Conversely, the number of European and Asian *T. pallidum* isolates identified using the ECDC and MLST methods was higher than that of American and African isolates. Therefore, molecular epidemiological studies are needed in the Americas to identify the major treponemal strains. The ECDC technique is critical for analyzing the epidemiology and clinical manifestations of syphilis owing to its discriminatory power, stability, and cost-effectiveness.

Antimicrobial Resistance in *Treponema pallidum*

Macrolides, which are a class of antimicrobials, are used to control and treat some human bacterial infections. The mechanism of macrolides, including azithromycin, involves binding to the 23S ribosomal RNA, inhibiting bacterial protein synthesis. Azithromycin is used to treat infections, such as pneumonia, sinusitis, pharyngitis, tonsillitis, and STIs (including gonococcal and chlamydial infections) (SALLE R, et al., 2024; WANG X, et al., 2023). Macrolide resistance in *T. pallidum* and failed clinical treatment of macrolide-resistant *T. pallidum* infections have been reported in several countries where macrolides were used as an alternative treatment for syphilis. Previous studies have demonstrated that the A2058G and A2059G mutations in the 23S rRNA gene confer macrolide resistance to *T. pallidum*. These mutations alter the antimicrobial-binding site, reducing therapy efficacy (TIECCO G, et al., 2021).

Epidemiological studies have revealed that the prevalence of macrolide resistance varies depending on the region. In China, high rates of A2058G mutation have been reported in Shanghai (95.4%), Hunan (97.5%), Shangdong (92.1%), and Xiamen (100%), while the A2059G mutation in the *T. pallidum* 23S rRNA gene has only been reported in Shangdong (WANG X, et al., 2023). Recently, the prevalence of macrolide-resistant *T. pallidum* has increased worldwide. In contrast, a genomic study in Australia revealed that 87% of *T. pallidum* samples were resistant to azithromycin (TAOUK ML, et al., 2022). The A2058G point mutation in the 23S rRNA gene of *T. pallidum* is the most frequent mutation associated with azithromycin resistance, while the A2059G mutation is uncommon (TIECCO G, et al., 2021).

However, the genetic origin and mechanisms underlying *T. pallidum* macrolide resistance are poorly understood. Penicillin-resistant treponemes have not been identified, and penicillin remains the only effective treatment for syphilis in pregnant women. Macrolide resistance has significant clinical implications, especially in settings where penicillin cannot be used. Decreased macrolide efficacy can lead to treatment failure and disease exacerbation. Additionally, emerging resistance can complicate efforts to control STIs, necessitating continuous monitoring and updated treatment guidelines (SALLE R, et al., 2024; WANG X, et al., 2023).

The resistance of *T. pallidum* to other antimicrobial classes, such as tetracyclines and cephalosporins is poorly understood. As penicillin-resistant *T. pallidum* strains have not been identified, penicillin remains the treatment of choice for syphilis. Antimicrobial resistance of *T. pallidum* has been poorly understood. Further studies are needed to elucidate resistance mechanisms, map the geographic distribution of resistant strains, and develop appropriate management strategies (SALLE et al., 2024; WANG X, et al., 2023). Ongoing genomic and epidemiological studies are crucial to monitor resistance evolution and modify clinical practices.

FINAL CONSIDERATIONS

Syphilis is a sexually transmitted bacterial infection that can be prevented and treated. However, the global increase in the incidence of this disease, particularly in Brazil, represents a growing public health concern. Early diagnosis of syphilis, in its initial stages, is essential for disease control, prevention of complications, and identification when it is most treatable. However, the efficacy of serological tests is limited, highlighting the need for developing novel diagnostic approaches. Molecular techniques are effective tools for diagnosing and understanding the transmission dynamics of *T*. *pallidum* infection. The application of PCR and molecular typing tests can improve early detection and infection monitoring and enhance our understanding of the epidemiological and genetic characteristics of *T*. *pallidum*. Limited data are available on *T*. *pallidum* characteristics and resistance status in Brazil. Analysis of resistance-related gene mutations is crucial for understanding disease evolution and its clinical implications.

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