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An update on the proteomic, molecular diagnosis and treatment of syphilis; A blood-transmitted disease

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ABSTRACT

Syphilis is a stimulating and disconcerting infection with diverse clinical symptoms and challenges in diagnosis and treatment. Its causative agent, *Treponema pallidum* (*T. pallidum*), is difficult to study due to its inability to be cultured or genetically modified. This study reviews recent advancements in molecular techniques that clarify the infection's biology, improve diagnostic tools, and assess treatment effectiveness with alternative antibiotics. In the past decade, research has expanded our understanding of syphilis pathogenesis, particularly the host immune response to *T. pallidum*. A key focus is its ability to evade immune responses, which hinders vaccine development. Antigenic variation in the TprK protein complicates vaccine progress, but ongoing studies are exploring conserved antigen regions for potential solutions.

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Introduction

Syphilis is an enthralling and confounding sexually transmitted disease, with protean clinical indices and both diagnostic and management uncertainties (Hook III & Marra, 1992). *Treponema pallidum* (*T. pallidum*) subsp. *Pallidum* bacterium, the agent of syphilis a challenging organism to study due to its inability to be subjected to culture in the laboratory or genetic modifications (Quétel et al., 1990). The spread of syphilis occurs through a variety of mechanisms, including sexual contact with infectious wounds, blood transfusions, or from an expectant mother to her fetus (Goh, 2005). Syphilis has been categorized into stages according to clinical results, facilitating the treatment and follow-up (Workowski & Berman, 2011). For effective treatment, individuals infected with syphilis might search for signs or indicators of primary syphilis infection (i.e., ulcers or chancres at the infection site), secondary syphilis (i.e., skin rash, lymphadenopathy and mucocutaneous lesions), or tertiary syphilis (i.e., cardiac issues, tabes dorsalis, gummatous lesions, and general paresis) (Workowski and Berman, 2010, Dionne-Odom et al., 2013). Infections that lack clinical signs (Latent infections) are identified by means of serologic testing. Latent syphilis picked up within the previous year is classified as early latent syphilis (Morshed & Singh, 2015). Every other case of latent syphilis is considered either late latent syphilis or syphilis of indefinite interval. The central nervous system is susceptible to *T. pallidum* infection, leading to neurosyphilis at any given stage of syphilis (Morshed & Singh, 2015, Ratnam, 2005). Primary neurologic clinical indices (i.e., cranial

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nerve dysfunction, acute altered mental status, meningitis, stroke and auditory or ophthalmic abnormalities) are typically existent in the first couple of months or years of infection (Brown & Frank, 2003). Late neurologic manifestations (i.e., tabes dorsalis and general paresis) arise 1 to 3 decades after acquiring the infection (Van Eijk et al., 1987).

Study design

This review consolidates comprehensive data from various studies on syphilis, offering a detailed overview of current insights into the disease. Over the past decade, significant advancements have enriched our understanding of syphilis, which are thoroughly explored in this work. The explored topics in this review article were summarized in figure 1.

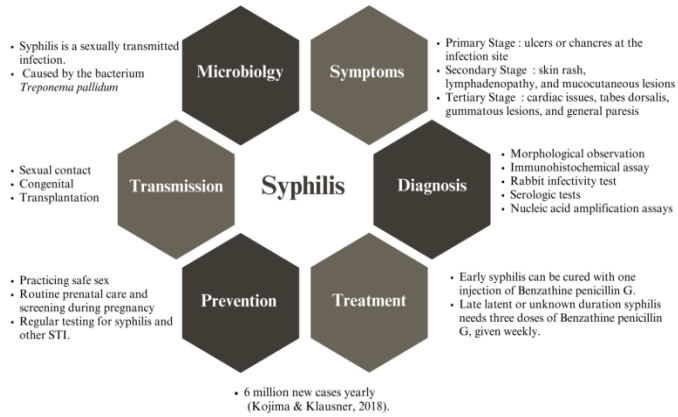

Fig 1. General overview of Syphilis

The underlying causes of syphilis

Syphilis is a chronic infectious disease caused by the spiral-shaped bacterium *T. pallidum* subspecies pallidum, part of the Spirochaetales order, which also includes the genera Borrelia and Leptospira (Peeling & Hook III, 2006). In addition to *T. pallidum* subspecies pallidum, which causes venereal syphilis, other pathogenic treponemes include *T. pallidum* subspecies pertenue (yaws) (Miao & Fieldsteel, 1978, Antal et al., 2002), *T. pallidum* subspecies endemicum (endemic syphilis), and *T. carateum* (pinta). The 'endemic' treponemes are morphologically identical to each other and to *T. pallidum* subspecies pallidum. They exhibit high DNA homology and close antigenic similarity but differ in geographic distribution, tissue specificity, animal infectivity, and pathogenesis (Centurion-Lara et al., 1998). Endemic treponematoses, unlike venereal syphilis, do not cause neurological disease or congenital transmission. They are typically acquired in childhood through non-sexual contact in communities with poor hygiene (Papp, 2024, Sankaran et al., 2023).

Molecular pathogenesis of syphilis

T. pallidum is a helical, micro-aerophilic bacterium measuring 6–20 µm in length and 0.10–0.18 µm in diameter. Its structure includes a central protoplasmic cylinder surrounded by a cytoplasmic membrane, a peptidoglycan layer, and an outer membrane (Cox et al., 1992). Two to three flagella located at each end controlling its motility. Unlike many bacteria, *T. pallidum's* outer membrane lacks lipopolysaccharides and has few surface-exposed transmembrane proteins, earning it the designation of a "stealth pathogen" due to its ability to evade immune detection (Salazar et al., 2002).

Recent studies have discovered the tpr gene family in *T. pallidum*, encoding proteins similar to the major surface proteins of *T. denticola*. These proteins play a role in host tissue attachment and function as porins (Centurion-Lara et al., 1999, Centurion-Lara et al., 2004). Tpr proteins are immunogenic in rabbits, with Tpr K being a target for opsonic antibodies that enable macrophages to clear treponemes. However, Tpr K has seven variable regions, and antibodies to these regions provide protection only against the same strain, not against different strains (Morgan et al., 2003; Leader et al., 2003).

Syphilis transmission

Sexual and vertical transmission

Sexual transmission occurs through direct contact with syphilis sores or chancres during vaginal, anal, or oral sex (Center, 2019). Chancres, characteristic of primary syphilis, appear on the external genitals, anus, vagina, rectum, or in or around the mouth. Sexual transmission can also occur during the secondary stage, primarily through direct contact with mucous membrane lesions, such as condyloma lata or mucous patches. Whereas, vertical transmission occurs when an infected mother transmits the infection to her unborn baby via the bloodstream (Center, 2019).

Epidemiology of syphilis in human

The prevalence of syphilis infection declined sharply after penicillin and other antibiotics were introduced in the 1940s. However, it began to increase again after human immunodeficiency virus (HIV) infection became more prevalent in the 1980s. The diverse occurrence of syphilis is shown by its higher prevalence in South of United States, in municipal areas, in men and blacks (Janier et al., 2014). Between 1990 and 2000, an 89.2% reduction in primary and secondary syphilis infection rates was observed. In 2019, 29 EU/EEA member States reported 35 039 confirmed syphilis cases, for a rough notification rate of

7.4 infections per 100 000 people (Control, 2022). The percentage of prevalence of syphilis in men was found to be nine times more likely than women, with rates highest in the male age 25-34 years, with 31 cases per 100 000 population (Control, 2022). About, 176,713 syphilis cases were recorded in 2021 which include both congenital syphilis and all syphilis stages, including 53,767 cases of primary and secondary syphilis cases which considered the most infectious syphilis stages (National Academies of Sciences and Medicine, 2021).

In spite of the general decline, syphilis infection outbreaks have been recently reported among sexually active gay males (Control and Prevention 2001, 2006), and according to the Centres for Disease Control and Prevention, the total rate of syphilis infection increased 27.5% during 2020 to 2021 (Tuddenham & Ghanem, 2022).

Epidemiology of syphilis in animals

Although treponemal illness is rare in animals, rabbit syphilis is a well-known clinical condition (Smith & Dobson, 1992). *Treponema paraluiscuniculi* bacteria are linked to rabbit syphilis, is distinct from that found in humans and cannot be transmitted from rabbit to human (Smith & Dobson, 1992). Rabbit syphilis is a low-grade treponematosis that is transmitted by direct touch, including coitus. The condition is mostly mucocutaneous and can last for months. The rabbits acquire vaginal lesions, which impair their reproductive ability, and they seem immunocompromised (Cunliffe-Beamer & Fox, 1981).

Also, researchers observed that wild African monkeys were naturally infected with *T, pallidum* in the 1960s and 1970s (Harper et al., 2012). Serological investigations in West Africa revealed that many baboon troops were seropositive with *T. pallidum*, with seroprevalence exceeding 60% in some parts of Senegal and Guinea (Fribourg-Blanc & Mollaret, 1969, Baylet et al., 1971). Clinical signs of infection include swollen of lymph nodes and tiny sores on the snout or near the armpit that contained high quantities of treponeme (Fribourg-Blanc et al., 1966).

Stages of syphilis

Acquired Syphilis (typically by sexual contact) is categorized into early and late syphilis. Early syphilis can be further classified into primary, secondary and early latent syphilis. The definition of early syphilis, also known as infectious syphilis, varies slightly between health organizations. In their definition, European Centre for Disease Prevention and Control (ECDC), refers to early syphilis as syphilis acquired less than one year earlier, while the World Health Organization (WHO) describes it as syphilis acquired less than two years ago. Different epidemiological and clinical considerations explain this difference, which doesn't impact the disease's diagnosis or treatment (Janier et al., 2014). Late syphilis consists of late latent and tertiary syphilis (gummatous, cardiovascular and neurosyphilis). The ECDC defines late syphilis as syphilis acquired>1 year prior to detecting the infection and WHO outlined it as syphilis acquired>2 years previously (Pao et al., 2002, Organization, 1999). A congenital syphilis infection can be classified into two stages: early (during the first two years of life) and late (after the first two years of life), comprising stigmata of congenital syphilis (Herremans et al., 2010).

Primary syphilis

It is most commonly related to a solitary, painless chancre at the site of infection; however, it is evident in other ways, such as numerous chancres, excruciating papules or ulcers, or absence of lesions (Brown & Frank, 2003, Tramont, 2005). The chancre is most frequently existent in the external genitalia and typically appears 10 days to 3 months (with an average of 21 days) after the initial infection (Luger, 1988). Primary syphilis is frequently associated with regional lymphadenopathy. The chancre typically heals naturally in 30 to 120 days. The ulcer is largely shallow, solitary, painless and indurated with no blistering exterior or a clean base expelling clear serum, mostly in the anogenital region. However, the lesions are regularly uncharacteristic in exterior and may be numerous, painful, cavernous, and indiscernible from herpes (Schroeter et al., 1971, Alexander et al., 1949, Schober et al., 1983). Any anogenital ulcer should be described as syphilitic except if it is not confirmed. In the case of anogenital ulcers, syphilis can be a cause; however, other conditions should be considered and ruled out before a diagnosis of syphilis can be made. Chancres are regularly hard to discover in women and men that sleep with men. Furthermore, preliminary tests for syphilis may not always provide a definitive result and an accurate diagnosis, and repeat serology testing at 1, 2, and 6 weeks may be necessary to confirm or exclude a diagnosis. In certain populations, delaying treatment can be detrimental, especially when patients are reluctant to return for follow-up and comprehensive examinations. (Schober et al., 1983).

Infection occurs when *T. pallidum* attaches to epithelial cells and extracellular matrix constituents of the skin and mucosa. A number of *T. pallidum* proteins are responsible for this process, including TP0155 and TP0483, which bind matrix and soluble/matrix fibronectin, respectively(Cameron et al., 2004). The

TP0136 protein is recognized by its typical reaction with primary human syphilitic sera (Brinkman et al., 2006), and its affixing to human fibronectin (Brinkman et al., 2008). TP0751 can adhere to laminin, which exhibits the maximum absorption in the basement membrane (Cameron, 2003, Cameron, 2007, Houston et al., 2011), and also to fibrinogen, a blood-clotting protein that works to hold bacteria (Houston et al., 2011). In addition, TP0751 also has a zinc-dependent protease domain that can degrade laminin and fibrinogen, which may allow *T. pallidum* to spread to nearby tissues and the blood stream (Houston et al., 2011).

The replication of *T. pallidum* at the location of preliminary inoculation undergoes a single division every 30-33 hours. (Hollander & Nell, 1954). This causes a discrete inflammatory response that leads to a painless chancre about 3–6 weeks following an initial infection. For every chancre, multiplying spirochetes are enclosed by immune cells, that include CD4+ and CD8+ T cells, plasma cells, and macrophages that produce IL-2 and IFN-γ cytokines, which are symptomatic of a Th1-skewed response (McBroom et al., 1999, Godornes et al., 2007, Leader et al., 2007). Tissue necrosis and ulceration arise as a result of minute vessel vasculitis, and transferring immune cells initiate a non-tender regional lymphadenopathy. In the course of 3 weeks to 2 months, the chancre heals, signifying the elimination of *T. pallidum* discretely. However, by this time, *T. pallidum* has systematically disseminated to various tissues and organs, leading to the development of secondary syphilis

Secondary syphilis

Within the first year, multisystem involvement stemming from bacteraemia can persist and may continue into the second year following the initial infection. In about 90% of syphilis cases, a non-itchy skin rash, called a roseola, develops 8-12 weeks after the onset of the chancre, followed by mucocutaneous lesions and, subsequently, papular syphilids.. Other possible outcomes include fever, generalised lymphadenopathy, hepatitis, splenomegaly, periostitis, arthritis, and glomerulonephritis (Farhi et al., 2009, Parc et al., 2007, Villanueva et al., 2000, Mishra & Tripathi, 2008, Ghanem et al., 2008). Meningitis, cranial nerve palsies, auricular and ophthalmic abnormalities (such as uveitis, retinitis, otitis and papillar edema), meningo-vascular syphilis (stroke, myelitis) can arise in secondary syphilis and should be customized as early neurosyphilis.

The symptoms of secondary syphilis usually emerge within 3 months of infection. The most frequently occurring clinical expression is a dispersed maculopapular rash. Additional symptoms may include malaise, weight loss, ocular inflammation, muscle pains, generalized lymphadenopathy, mucous patches, meningitis, (localized inflammation of mucosal tissues in the oral cavity and genitals), hepatitis, and gastric dysmotility (Chapel, 1980, Mindel et al., 1989), which are symptomatic of *T. pallidum* incursion and the resultant immune cell invasion of these tissues. According to electron microscopic micrographs of secondary syphilis skin abrasions, *T. pallidum* possibly utilizes transcytosis to proliferate through the endothelium (Juanpere-Rodero et al., 2013). *T. pallidum* can initiate the generation of MMP-1 (Chung et al., 2002), which causes the degradation of collagen and possibly enables entry into and exit from the bloodstream, leading to complete dissemination.

Latent syphilis

This stage entails positive serological examinations for syphilis in the absence of clinical proof of treponemal disease. It is randomly categorized as early if it occurs within the first year and late after >1 year (or uncertain period). Early latent syphilis comprises individuals that tested positive when administered the serological tests for syphilis, a negative serological tests for syphilis within a year of a diagnosis or a fourfold (2 dilutions) or a significant reduction of Non-treponemal antibodies titre or unambiguous proof that the patients were infected in the previous year (based on the clinical symptoms in patient and cohorts) (Wharton et al., 1990).

Tertiary syphilis

This stage of syphilis includes gummatous syphilis (nodules/plaques or ulcers (skin, mucosae, visceral), late neurosyphilis (meningitis, cranial nerve dysfunction, meningo-vascular syphilis (stroke, myelitis) and parenchymatous neurosyphilis (general paresis, tabes dorsalis)); cardiovascular syphilis (aortic regurgitation, stenosis of coronary ostia, aortic aneurysm (mainly thoracic)); neurologic syphilis (meningitis, cranial nerve dysfunction, can arise early (secondary syphilis) or late (tertiary syphilis)). Gummas manifest as granulomatous-like lesions that are clinically substantial since they instigate confined destruction (Tramont, 2005). Although any organ can be affected by these lesions, the bones, skin, and mucous membranes are most commonly affected.. Cardiovascular syphilis develops due to the destruction of the elastic tissue in the aorta, leading to aortitis and the formation of aneurysms, which rarely rupture. The most regularly affected is the ascending aorta, associated with the possible

complexities of aortic valve inadequacy and coronary artery stenosis. A diagnostic indication of cardiovascular syphilis is the presence of linear calcifications of the aorta on cardiovascular radiographs. About 11% of patients who do not receive treatment progress to cardiovascular syphilis (Clark & Danbolt, 1964). The antibiotic treatments for gummatous, cardiovascular and late latent syphilis are similar, on condition that there is no proof of neurologic contribution. However, there is no consensus on appropriate follow-up for tertiary syphilis patients without CNS involvement. Clinical response to treatment differs and is dependent on the kind and site of gummatous or cardiovascular lesions.

Diagnosis of syphilis:

Various methods for syphilis diagnosing are outlined in figure 2.

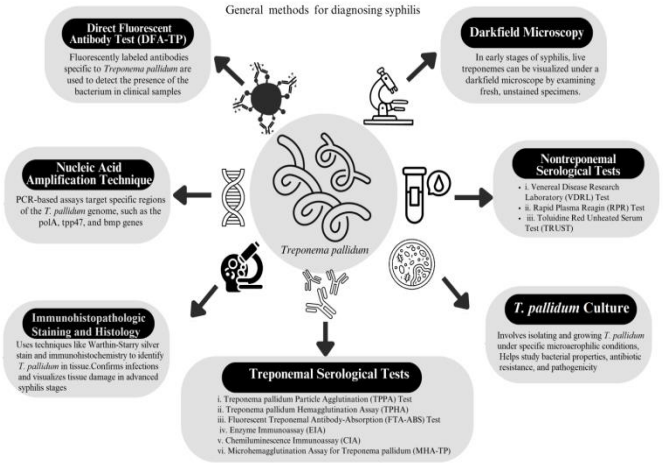

Fig 2. Different techniques for diagnosis of syphilis.

Dark-field microscopy

Dark-field microscopy is the most definite method for syphilis diagnosis in the case of active chancre or condyloma latum (Larsen et al., 1995). However, its precision is limited by a number of factors that include the capability of the operator that performs the examination, the amount of live treponemes in the lesion, and the incidence of non-pathologic treponemes in oral or anal lesions (Cummings et al., 1996). The dark-field microscopy preparation involves the washing of the lesions followed by mild scraping with a gauze pad. Once a serous exudate emerges, it is mounted on a glass slide and observed under a microscope coupled with a dark-field condenser (Larsen et al., 1995). *T. pallidum* is recognized by its distinctive corkscrew exterior. Based on the intrinsic problems of dark-field microscopy, negative examinations on three diverse days are essential prior to considering a lesion is negative for *T. pallidum* (Tramont, 2005).

Non-treponemal tests

Syphilis infection stimulates the production of generic antibodies that are reactive to cardiolipin, a component of host tissues that are damaged during infection. These antibodies form the basis of conventional nontreponemal tests, including the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin tests (RPR). The pregnancy, autoimmune disorders, and infections account for possible false-positive reactions in nontreponemal tests (Luger, 1988, Fischbach, 2000, Larsen et al., 1995). Furthermore, these assessments may display a "prozone" singularity where enormous volumes of antibody impede the antibody-antigen reaction, resulting in a false-negative test in the sample prior to dilution (Larsen et al., 1995). Qualitative nontreponemal tests are extensively utilized for the screening of syphilis infection. Nonetheless, their efficacy is restricted by reduced sensitivity in early primary syphilis and during late syphilis, when approximately one third of untreated patients may be inert (Cummings et al., 1996). Following the sufficient syphilis treatment, the nontreponemal tests ultimately become nonreactive. After effective treatment for syphilis, the nontreponemal tests eventually become negative. However, some patients may still have a persistent low-level positive nontreponemal test, known as a serofast reaction, despite adequate treatment. It is important to note that titers cannot be compared between different test methods, and the same nontreponemal test used for the initial diagnosis should be used for follow-up assessments (Papp, 2024).

Treponemal-specific tests

Treponemal-specific tests identify antibodies to antigenic constituents of *T. pallidum*. These tests are primarily used as reactive nontreponemal tests to confirm the diagnosis of syphilis in patients (Papp, 2024). Additionally, the enzyme immunoassay (EIA) test for anti-treponemal IgG can be used for screening (Young & Gorski, 1995). Treponemal-specific tests comprise the EIA for anti-treponemal IgG, the *T. pallidum* hemagglutination (TPHA) test, the microhemagglutination test with *T. pallidum* antigen, the fluorescent treponemal antibodyabsorption test (FTA-abs), and the enzyme-linked immunosorbent assay. The sensitivities and specificities of treponemal tests are equivalent to or exceed those of nontreponemal tests (Larsen et al., 1995). Nonetheless, treponemal-specific screening tests are more complex and costly to

carry out, which constrains their practicality. Furthermore, the conundrum of false-positive results may arise, particularly in cases where FTA-abs test is utilized in patients afflicted with Lyme disease or systemic lupus erythematosus (Larsen et al., 1995, Carlsson et al., 1991). Contrary to nontreponemal tests, which display a reduction in titers or become inert with efficacious treatment, treponemal-specific tests typically stay reactive permanently. Thus, treponemal-specific test titers are not valuable for evaluating the effectiveness of syphilis treatment.

Molecular strain typing of T. pallidum

A technique for distinguishing one strain of *T. pallidum* from others is necessary to obtain vital data on the transmission of syphilis infection, and particularly to understand the development and dissemination of antibiotic-resistant strains.. Pillay et al., designed a *T. pallidum* typing technique according to (a) quantification of the amount of 60-bp repeats in the acidic repeat protein (arp) gene and (b) sequence disparities in the Tpr subfamily II genes (tprE [tp0313], tprG [tp0317], and tprJ [tp0621]) measured by restriction fragment length polymorphism (RFLP) analysis (Pillay et al., 1998). *T. pallidum* subtypes with a series of 2 to 21 ARP repeats and 7 dissimilar RFLP patterns, labelled a–g, were described (Pillay et al., 1998). This technique for subtype designation has been implemented on patient samples obtained from chancres, condyloma lata, scrapes from the mouth and ear lobe, blood tissue, CSF, and laboratory-passaged *T. pallidum* isolates from different geographical locations (Pillay et al., 1998; Sutton et al., 2001, Pillay et al., 2002, Pope et al., 2005; Molepo et al., 2007; Florindo et al., 2008; Castro et al., 2009; Cole et al., 2009; Martin et al., 2009). Epidemiological research of strain types in San Francisco and Seattle in the past 10 years indicated that the majority are subtype 14d (Katz et al., 2010; Marra et al., 2010), which possibly implies a connected sexual network, whereas other studies point to location disparity in the distribution of major strain types in the United States and globally (Sutton et al., 2001; Pillay et al., 200; Pope et al., 2005; Molepo et al., 2007; Cole et al., 2009; Martin et al., 2009). The incorporation of a third gene to the typing scheme amplified the differentiating power (Katz et al., 2010; Marra et al., 2010). The use of tp0548 gene for subtyping proved that the major 14d/f strain was substituted by the 14d/g strain in Seattle for the duration of 1999 to 2008 (Marra et al., 2010). This identification process cannot be accomplished using the two-target typing method.

In addition, molecular analysis is utilized to define whether macrolide- resistant *T. pallidum* denotes a solitary strain or if resistance has suddenly stemmed from numerous strains. The A2058G mutation was discovered in molecularly distinct strains in Seattle, signifying that resistance mutations arise freely, instead of signifying a solitary strain dispersing all over a populace. The probability of resistant strains occurring is higher in patients who had garnered macrolide antibiotics in the last 1 year (Katz et al., 2010; Marra et al., 2010). In addition, strain typing is possibly utilized to detect strains related to specific medical outcomes. Medical reports from the pre-antibiotic time and research in the rabbit infection model indicate that certain strains exhibit a higher predisposition to neuroinvasion (Tantalo et al., 2005). A contemporary study of *T. pallidum* revealed that patients diseased with strain type 14d/f exhibited a higher rate of neurosyphilis in contrast to patients ill from other strain types (Marra et al., 2010).

T. pallidum culture

T. pallidum cannot be grown using standard laboratory culture media (SeÑa et al., 2015). Rabbit infectivity test, which isolates *T. pallidum* by inoculating a clinical specimen into the testes of live rabbits, is the only available method for its isolation. However, it is not suitable for routine diagnosis due to its high time and cost demands, the need for specialized personnel, and ethical concerns related to the use of live animals (SeÑa et al., 2015).

Nucleic acid amplification testing

Since *T. pallidum* cannot be cultured on standard media, molecular assays, such as nucleic acid amplification tests (NAATs), are used to directly detect *T. pallidum* DNA, enhancing diagnostic sensitivity (Satyaputra et al., 2021). NAAT methods include PCR, nested PCR, quantitative PCR, and reverse transcriptase PCR (Theel et al., 2020). The most commonly used and studied target genes are the *T. pallidum* 47-kDa lipoprotein (tpp47) and the DNA polymerase A gene (polA) (Grange et al., 2012).

Additional target genes include treponemal membrane protein A (tmpA), subsurface lipoprotein 4D (4D), and basic membrane protein (bmp). These assays are not yet commercially available (Peeling et al., 2017).

Histology and immunohistopathologic staining

Syphilis produces histological changes in affected tissues that vary based on the lesion type and disease stage. Primary syphilis is typically marked by a chancre, with histology revealing an acanthotic epidermis that

ulcerates over time, dense infiltration of lymphocytes and plasma cells, and endothelial swelling (Groh et al., 2014). Spirochetes are often detected in or around blood vessels and at the dermal-epidermal junction. The histological features of secondary syphilis are highly variable; the epidermis may appear normal, necrotic, or ulcerated, while the dermis may exhibit changes similar to primary syphilis, including dense lymphocytic infiltration and endothelial swelling (Solnick et al., 2024).

Tertiary syphilis is marked by localized inflammation or the formation of necrotizing granulomatous lesions (gummas) in tissues such as the skin, bones, and aorta (Groh et al., 2014). Silver and histological stains can be used to visualize spirochetes in formalin-fixed, paraffin-embedded tissue from primary and secondary syphilis lesions, but they are ineffective for detecting spirochetes in tertiary syphilis (Van Den Heuvel et al., 2019). Immunohistochemistry staining provides greater sensitivity and specificity compared to silver staining (Brischetto et al., 2018).

Diagnostic considerations of primary syphilis

The decisive method for the diagnosis of early syphilis is the use of Dark field examinations for the detection of *T. pallidum* directly from the mass of cells and fluids that seep from lesions or tissues (Marrazzo, 2013). Even though there are no commercially obtainable *T. pallidum* detection examinations, certain laboratories offer locally established and certified PCR tests for the detection of T. pallidum DNA. A probable diagnosis of syphilis needs two tests: a nontreponemal test (i.e., [VDRL] or Rapid Plasma Reagin [RPR]) and a treponemal test (i.e., fluorescent treponemal antibody absorbed [FTA-ABS] tests, the *T. pallidum* passive particle agglutination [TP-PA] assay, several enzyme immunoassays [EIAs], chemiluminescence immunoassays, immunoblots, or rapid treponemal assays). Despite the commercial availability of several treponemal-based tests, only a limited few have been approved in the different countries. The utilization of only a single kind of serologic test is inadequate for diagnosis and can lead to false-negative results in individuals examined during primary syphilis and false-positive outcomes in syphilis free individuals. False-positive nontreponemal test results are related to various health disorders and causes unconnected to syphilis, such as HIV, autoimmune conditions, immunizations, pregnancy, injection-drug use, and older age (Marrazzo, 2013, Nandwani and Evans, 1995). Individuals with a reactive nontreponemal test should therefore be given a treponemal examination continuously to confirm the diagnosis of syphilis. Nontreponemal test antibody titters are associated with disease activity and are utilized to track reactions to treatment. It requires the quantitative reporting of results. A fourfold amendment in titter, equal to an alteration of two dilutions (e.g., from 1:16 to 1:4 or from 1:8 to 1:32), is regarded as essential to validate a clinically significant disparity between two nontreponemal test results acquired with the same serologic test. Sequential serologic tests in specific patients are required to be done with similar testing techniques (VDRL or RPR), possibly by the same test center. The VDRL and RPR are similarly valid assays, although quantitative outcomes from the two tests cannot be comparatively analyzed directly since RPR titers often slightly exceed VDRL titers. Nontreponemal test titers typically decrease after treatment and might become inert with time; though, in certain individuals, nontreponemal antibodies can persevere for a lengthy duration, a response denoted as the “serofast reaction”. The reactive treponemal tests of the majority of patients will remain so for the rest of their lives, irrespective of treatment or disease activity. Nonetheless, 15%–25% of patients subjected to treatment in the primary stage are returned to being serologically nonreactive after 24 to 36 months (Romanowski et al., 1991). Treponemal antibody titers do not forecast treatment responses and thus should not be utilized for this purpose.

Some scientific laboratories screen samples using treponemal tests, normally using immunoassays with EIA or chemiluminescence (Control & Prevention, 2011; Workowski & Berman, 2011). A reverse screening algorithm for syphilis diagnosis will identify people who have been previously diagnosed for syphilis, those with untreated or partially treated syphilis, and individuals with false-positive outcomes that can arise with a low probability of infection. People with a positive treponemal screening test should undergo a regular nontreponemal test with titter carried out reflexively by the laboratory to direct patient administration choices. For a negative nontreponemal test, the test center should conduct a different treponemal test (possibly based on diverse antigens compared to the initial test) to validate the outcomes of the original test (Workowski & Berman, 2007). For a positive second treponemal test, individuals with past treatment will need no additional management except when sexual history indicates the possibility of re-exposure. In this case, a replication of nontreponemal test in 2–4 weeks is recommended for early infection assessment. In the absence of a history of syphilis, the person should be provided treatment. Except in cases where history or outcomes of a physical examination implies a current infection, hitherto untreated persons

should be subjected to treatment for late latent syphilis. In the case of negative second treponemal test and low epidemiologic risk and clinical probability for syphilis, additional assessment or treatment is not requisite. Two earlier researches have shown a correlation between high quantitative index values from treponemal EIA/CIA tests and TPPA positivity. Nonetheless, the array of optical density values differs among diverse treponemal immunoassays, and the scientific implication of these results require additional study (Park et al., 2011; Wong et al., 2011).

For majority of individuals infected with HIV, serologic tests are precise and dependable for the diagnosis of syphilis and monitoring a patient's response to treatment. Nonetheless, unusual nontreponemal serologic test results (i.e., abnormally high, abnormally low, or inconsistent titters) might arise irrespective of HIV-infection status. In cases where serologic tests do not match clinical findings indicative of early syphilis, presumptive treatment is suggested for high-risk individuals, and additional tests such as biopsy and PCR should be taken into consideration.

Additional testing is necessary for individuals with medical signs of neurosyphilis, which include stroke, auditory or ophthalmic abnormalities, acute or chronic altered mental status, meningitis, cranial nerve dysfunction, and loss of vibration sense. Laboratory testing is valuable in supporting neurosyphilis diagnosis; however, no particular test can be utilized to diagnose neurosyphilis in all cases. The diagnosis of neurosyphilis is dependent on a mixture of cerebrospinal fluid (CSF) tests (CSF cell count or protein and a reactive CSF-VDRL) in conjunction with reactive serologic test outcomes and neurologic signs and symptoms. CSF laboratory abnormalities frequently occur in individuals with early syphilis and are of indefinite implication in the absence of neurologic signs or symptoms (Lukehart et al., 1988). CSF-VDRL is extremely precise but insensitive. Thus, in a person with neurologic signs or symptoms, a reactive CSF-VDRL (in the lack of blood infection) is considered indicative of neurosyphilis. In the case of negative CSF-VDRL, regardless of the occurrence of clinical signs of neurosyphilis, reactive serologic test results, abnormal CSF cell count and/or protein, and neurosyphilis should be considered. In this case, further assessment by means of FTA-ABS testing on CSF may be acceptable. The CSF FTA-ABS test is less precise for neurosyphilis compared to CSF-VDRL, although it is highly sensitive. Neurosyphilis is very implausible with a negative CSF FTA-ABS test, particularly amongst people with generic neurologic signs and symptoms (Jaffe et al., 1978). CSF leukocyte count is typically high (>5 white blood cell count [WBC]/mm3) amongst HIV infected individuals. The use of a higher cut-off (>20 WBC/ mm3) might enhance the specificity of neurosyphilis diagnosis (Marra et al., 2004).

Treatment

Treatment procedures

Guidelines from the Centers for Disease Control and Prevention (CDCP) advocate the controlled administration of penicillin G for the treatment of all phases of syphilis (Control and Prevention, 2002, Workowski & Berman, 2011). Different regimens may be used for penicillin allergic patients. Nonetheless, pregnant women and patients with neurosyphilis need penicillin treatment despite their possible drug allergy. Hence, desensitization in these patients is necessary, prior to initiating the penicillin therapy. Syphilis treatment is similar in both HIV-positive and HIV-negative patients. However, HIV-positive patients need more regular follow-up due to an elevated risk of treatment failure. Furthermore, a higher index of suspicion for central nervous system (CNS) involvement must be sustained (Control and Prevention, 2002). Syphilis treatment at any stage should take into consideration the threats of acquiring other STDs. HIV examination should be considered in the preliminary assessment of all patients with syphilitic infection (Control and Prevention, 2002). Screening for hepatitis B and *C, gonorrhoea*, and chlamydial diseases should also be taken into account.

Penicillin G, which is parenterally administered, is the favoured drug for syphilis treatment at all stages. The preparation procedure employed (i.e., benzathine, aqueous procaine, or aqueous crystalline), dosage, and duration of treatment are dependent on the stage and clinical indices of the disease. Treatment for late latent syphilis and tertiary syphilis require a lengthier period of therapy, due to the theoretically slow division of the organisms (the rationality of this logic has not been evaluated). Nonetheless, extended treatment duration is requisite for individuals with latent syphilis of indefinite duration to guarantee that those who were not infected with syphilis the previous year are sufficiently treated.

Choosing the right penicillin preparation is vital, since *T. pallidum* can infect sites that are difficult to access with certain types of penicillin, such as the central nervous system and aqueous humor of the eye.. Blends of benzathine penicillin, procaine penicillin, and oral penicillin preparations are not regarded as suitable for syphilis treatment. Studies have shown that practitioners have involuntarily prescribed the combination of benzathine-procaine penicillin (Bicillin C-R) rather than the standard benzathine penicillin

product (Bicillin L-A) that is extensively utilized in the US. Practitioners, pharmacists, and buyers should be cognizant of the similarity of the names of these two products to circumvent using the incongruous combination therapy agent for syphilis treatment (Control and Prevention, 2005).

The efficacy of penicillin for syphilis treatment is proven and reputable through clinical experience even before the significance of arbitrarily controlled clinical trials was acknowledged. Consequently, almost all recommendations for syphilis treatment are centred on experimental trials, observational studies and several years of clinical experience. After administering appropriate treatment, the follow-up action should involve measuring quantitative nontreponemal test titers to assess treatment response. Nontreponemal test titers typically decrease by at least fourfold within six months after treatment of primary or secondary syphilis. In cases of latent syphilis or late infection, it may take one to two years after treatment for nontreponemal test titers to similarly decrease (Romanowski et al., 1991).

Special considerations situations

Pregnancy

Parenteral penicillin G is the only known effective treatment for syphilis during pregnancy. Pregnant women with syphilis who are allergic to penicillin should undergo penicillin desensitization therapy as soon as possible (Control and Prevention, 2016).

Jarisch-Herxheimer Reaction

The Jarisch-Herxheimer reaction is an acute febrile reaction often followed by headache, muscle pains, fever, and other symptoms that can arise within a day after the start of any treatment therapy for syphilis. Patients should be informed of potential adverse reactions and ways to manage them if they arise. The Jarisch-Herxheimer reaction occurs most commonly among persons infected with early syphilis, apparently due to higher bacterial burdens during this stage. Antipyretics can be utilized to cope with symptoms, although their preventive capabilities have not been confirmed. The Jarisch- Herxheimer reaction might induce early labour or cause foetal distress in pregnant women, however, this should not prevent or delay treatment for syphilis (Genç & Ledger, 2000) .

Management of sex partners

The sexual transmission of *T. pallidum* is believed to arise simply when mucocutaneous syphilitic lesions are existent. Such indicators are infrequent following the first year of infection. Individuals who have unprotected sex with those infected with primary, secondary, or early latent syphilis need to be assessed clinically and serologically, and treated based on the underlying recommendations:

- Individuals who have engaged sexually with persons diagnosed with primary, secondary, or early latent syphilis 3 months prior to the diagnosis should be subjected to presumptive treatment for early syphilis, despite testing negative serologically.
- Individuals who have sexually engaged with those diagnosed with primary, secondary, or early latent syphilis >3 months preceding the diagnosis should be treated presumptively for early syphilis if serologic test results are not immediately obtainable and the chance for subsequent treatment is indefinite. However, if serologic tests are positive, treatment should be based on clinical and serologic evaluation and the stage of syphilis. No treatment is necessary if serologic tests are negative..
- In some places or populations with significant syphilis incidences, health departments strongly recommend notifying and presumptively treating for sex partners of individuals with late latent syphilis and high nontreponemal serological test titres (i.e., >1:32), since high titters indicate early syphilis symptoms. These cohorts should be handled as if the index case is infected with early syphilis.
- Long-standing sex partners of individuals infected with late latent syphilis should be assessed clinically and serologically for syphilis and subjected to treatment based on the results.

Those who have had sexual contact with a syphilis patient should be informed privately of their exposure. The notification period varies depending on the type of syphilis: 1) for those with primary syphilis, sexual partners from the past 3 months should be notified, along with the duration of symptoms manifestations; 2) for those with secondary syphilis, sexual partners from the past 6 months should be notified, along with the duration of symptoms manifestations; and 3) for those with early latent syphilis, sexual partners from the past 12 months should be notified.

Women with pregnancy

Congenital syphilis, mother-to-child transmission of syphilis, is commonly distressing to the foetus if maternal infection is undetected and inadequately treated early on in pregnancy. The susceptibility to morbidity and mortality because of congenital syphilis is high. In 2012, a projected 350 000 adverse pregnancy outcomes globally were attributed to syphilis,

comprising 143 000 early foetal deaths/stillbirths, 62 000 neonatal deaths, 44 000 preterm/low-birth-weight babies and 102 000 infected infants (Mishra and Tripathi, 2008, Mishra, 2019). Majority of pregnant women encumbered by untreated primary and secondary syphilis infections are at a higher risk of experiencing. Over half of pregnant women infected with latent (asymptomatic) syphilis are hindered by adverse pregnancy outcomes. Nonetheless, the foetus can be easily alleviated with treatment, and the possibility of adverse consequences to the foetus is marginal if the mother receives sufficient treatment during early pregnancy – preferably prior to the second trimester.

All expectant mothers should be subjected to serologic test for syphilis during the first prenatal visit. In a case where access to optimal prenatal care is unavailable, alternative examinations such as rapid plasma reagin (RPR) card test screening (and treatment, if that test is reactive) should be deployed when a pregnancy is confirmed (Sheffield et al., 2007). Women who are highly susceptible to syphilis or reside in areas of high preponderance of syphilis morbidity should be screened once more early in the third trimester (about 28 weeks' gestation) and also at delivery. Certain places demand the screening of every woman at delivery. It is highly recommended that neonates should not be cleared from the hospital except the syphilis serologic status of the mother has been determined a minimum of one time in the course of pregnancy and preferably once more at delivery for high risk cases. Also, women who delivered stillborn babies should be subjected to syphilis testing (Mishra, 2019).

Persons in prison or correctional facilities

Screening tests should be performed globally according to the local area and institutional incidence of early (primary, secondary, and early latent) contagious syphilis. Nonetheless, correctional facilities should remain cognizant of the prevalence of syphilis, given that it varies with time.

Men Who Have Sex with Men

An estimated two-thirds of the diagnosed cases of primary and secondary syphilis infections in the United States occur within the MSM populace, specifically among those in minority ethnic groups (Chesson et al., 2010, Patton et al., 2014). Improved syphilis screening procedures among MSM have led to an expansion of early syphilis detection; nonetheless, 71% of the syphilis diagnoses were confirmed when the patient searched for care to treat symptoms (Kerani et al., 2007). Severe HIV infection has been linked to STDs, including syphilis, among homosexuals at public STD treatment centres (Zetola et al., 2009), and in the multi-complex iPrex study (Solomon et al., 2014). In addition, a number of reports have shown that early syphilis infection is associated with HIV among MSM (Paz-Bailey et al., 2004, Pathela et al., 2011).

Factors related to rise in syphilis prevalence among MSM have included drug abuse (e.g., methamphetamine), having numerous unknown sexual partners, and searching for sex partners online (Chew Ng et al., 2013, Bernstein et al., 2013). A study reported 5.9% of MSM contracted primary or secondary syphilis infection repeatedly within 2 years of an original infection. The factors linked to recurrent syphilis infection were HIV infection, race (black) and having sex with 10 or more current sexual partners (Cohen et al., 2012). Given the risk of repeat infection, data indicate that deterrence efforts should include follow up serologic testing. Syphilis serology should be conducted a minimum of once a year for MSM that actively indulge in sexual practices, to determine individuals with untreated syphilis, incompletely treated syphilis, and those displaying a slow serologic response to suitable therapy, or are serofast.

Association between HIV and syphilis

From the onset of HIV/AIDS prevalence, there has been a reported high rate of HIV-1 (HIV) coinfection among syphilis patients. In 2002, the CDC stated that 25% of primary and secondary syphilis cases occurred in persons coinfected with HIV, and the prevalence rate of syphilis in HIV-infected persons was 77 times greater compared to the overall populace (Chesson et al., 2005). The incidents of early syphilis have continually risen over the last 10 years globally, predominantly in men who have sex with men (MSM), and in the last half decade in both black American males and females (Workowski and Berman, 2010). Although the rising prevalence of syphilis may be attributed to high risk tendencies (Workowski and Berman, 2007, Spindler et al., 2007, Taylor et al., 2007), the higher coinfection rates of syphilis and HIV may also be ascribed to immunological and bacteriological influences.

The contracting and transmission of HIV is enabled by primary chancre via the disruption of mucous membrane and epithelial tissue barriers (Greenblatt et al., 1988). Furthermore, the incursion of lymphocytes, neutrophils and monocytes into syphilis lesions raises the amount of cellular targets accessible for HIV infection and the contiguity of HIV-infected cells to spread virus to the proximate *T. pallidum* and *T. pallidum* lipoproteins. This, in turn, upregulates the expression of CCR5, a chemokine receptor that is

expressed on large phagocytic cells (macrophages) and dendritic cells (DCs), and serves as a co-receptor for HIV entry into CD4+ cells (Sheffield et al., 2007). However, it is not apparent if HIV coinfection hinders the clinical indicators of early syphilis or neurosyphilis. Nonetheless, clinical and CSF anomalies in accordance with neurosyphilis are more frequently found in HIV-infected individuals with CD4+ T cell counts lower than or equivalent to 350 cells/ml (Ghanem et al., 2009). Individuals co-infected with neurosyphilis and HIV possess elevated CSF HIV RNA concentrations, implying the possibility of an interaction between syphilis and HIV in the central nervous system (de Almeida et al., 2010). Although the long-standing effects of syphilis disease on the diagnosis of HIV-infected individuals are not fully understood(Weintrob et al., 2010), a study reported that syphilis does not seem to influence HIV advancement, despite the ephemeral proliferations in CD4+ T cell counts and viral loads. Nonetheless, *T. pallidum* coinfection is reported to have a lethal effect on the immunologic and virologic status of HIV-infected persons, which may improve with syphilis treatment, although these data are inconsistent (Sadiq et al., 2005, Buchacz et al., 2004, Kofoed et al., 2006, Palacios et al., 2007).

Vaccine advancement and deterrence

Despite the availability of highly efficacious syphilis treatment, there is presently an epidemic of syphilis in major parts of the world including China, with an increasing prevalence in the United States and Europe. Thus, the most effective tentative solution to control the proliferation of syphilis is through developing immunization against both infection and transmission. Efforts have been made over several years to manufacture an efficacious syphilis vaccine by testing on animals (Lukehart, 1985, Cullen and Cameron, 2006).

Early on, Miller (1973) performed an immunization study using numerous intravenous doses of gamma-irradiated *T. pallidum*, which validated complete protection against syphilis infection. However, this protocol was very difficult to implement and costly, and unfeasible to experiment in humans. Subsequent studies have demonstrated that immunization with recombinant *T. pallidum* antigens can induce an immune response in rabbits model, resulting in limited protection and considerably attenuated lesion growth. However, this approach does not provide sterile immunity (Centurion-Lara et al., 1999, Morgan et al., 2002, Cameron et al., 1998, Cameron et al., 2000, Sun et al., 2004, Giacani et al., 2005). The unearthing of antigenic variation in TprK makes the development of a vaccine even more daunting. However, studies are currently being performed to test the ability of a mélange of conserved regions of *T. pallidum* antigens.

Conclusion

The prevalence and proliferation of syphilis have been extensively studied, and despite the accessibility to cost-effective and efficacious treatment, the disease is gradually progressing in several countries. *T. pallidum* has been confirmed to be a perplexing infectious agent to study due to its physical instability, its outbred animal model, and its inability to be cultured or genetically modified. Despite these challenges, highly discerning molecular methods have been developed for strain differentiation and to target intervention activities, with the aim of elucidating the proliferation of the syphilis infection. This review provides a detailed insight into the current understanding of the molecular pathogenesis of syphilis, which has significantly advanced in the past 15 years, particularly in explaining the host immune response to *T. pallidum*. Innovative solutions have been developed such as the discovery of antigenic variation in TprK, which makes the advancement of a protective vaccine even more challenging. Research on this intriguing infection continues to emphasize on understanding its ability to elude host immune responses, which may eventually result in the development of an efficacious vaccine.

Conflict of interest

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Data availability

All data obtained from this study are included in the present manuscript.

Ethical statement

Not applicable.

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