Mini-review: Syphilis

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Syphilis is a complex multistage disease responsible for a range of severe clinical manifestations when untreated or as a result of treatment failure in its sole human host. The review briefly explores the complex biology of *Treponema pallidum* subsp. *pallidum*, the causative agent, and its successful pathogenesis as well as the techniques commonly employed for the detection of this non-culturable pathogen. Antimicrobial resistance to second-line antibiotics of *T. pallidum* subsp. *pallidum* is also discussed as an important evolutionary advantage conferred on this venereal pathogen.

Keywords *Treponema pallidum*; antimicrobial resistance; molecular detection

1. Introduction

Syphilis has plagued the human race since the 15th century [1, 2]. The aetiological agent *Treponema pallidum* subsp. *pallidum* (*T. pallidum)* is a spirochaete, which resembles a Gram-negative bacterium; however, differs with regard to a paucity of surface exposed outer membrane proteins capable of eliciting a host response and has demonstrated the ability to evade the host immune system effectively by the process of antigenic variation [3-6]. Despite a small genome and being metabolically limited compared to other bacterial pathogens, *T. pallidum* is regarded as a successful pathogen with several attributes involved in attachment, motility, invasion and immune-evasion to allow establishment of infection and clinical disease manifestations [6-9]. *Treponema pallidum* is responsible for a chronic multistage disease, which can present a myriad of clinical complications when untreated, such as neurosyphilis, congenital syphilis, gummas and cardiovascular syphilis [6].

Penicillin has been used successfully for the treatment of syphilis for over 50 years but the need for intramuscular administration and patient hypersensitivity to penicillin has led to the development and use of second-line antimicrobials [10, 11]. Selective pressure due to overuse of these agents may have contributed to the observed increase in antibiotic resistance [10, 11]. The challenge of phenotypic susceptibility testing remains as *T. pallidum* cannot be cultured on routine bacteriological media and only a few generations have been propagated in rabbit epithelial cell monolayers at a time [5, 12]. Detection of the organism relies mainly on serology and in some instances dark-field microscopy can be used especially in scrapings from genital ulcers [13-15]. New molecular techniques, however, could compliment conventional detection techniques, especially for the detection of antibiotic-resistant strains.

Given that *T. pallidum* is a sexually transmitted pathogen affecting individuals worldwide, with an estimated 12 million new cases annually, importance is placed on the rapid diagnosis, management and treatment of the aetiological agent [16]. In developing countries high human immunodeficiency virus (HIV) prevalence highlights the importance of syphilis co-infections with HIV positive individuals because of the implication it has in increasing the transmission of both venereal diseases and the altered malignant progression of the disease observed in immunocompromised individuals [17, 18]. Vaccine development for syphilis has had pitfalls and not proved successful despite the research done over the years [19]. The aim of the review is thus to summarise the biology of *Treponema pallidum* subsp. *pallidum* with a focus on antimicrobial resistance.

2. Epidemiology of *Treponema pallidum* subsp. *pallidum*

Syphilis is a multistage disease that has no geographical boundaries but of which a disproportionate amount of up to 90% occurs in developing countries [16, 20]. The World Health Organization (WHO) estimated that the majority of 12 million annual new cases occur in Africa, South and Southeast Asia, Latin America and the Caribbean [16, 21]. *Treponema pallidum* is predominantly a sexually transmitted pathogen but the risk of vertical transmission remains a concern due to its devastating effects [5, 12]. Congenital syphilis can be of particular concern in developing countries where prenatal diagnosis and treatment of pregnant women may be lacking [5]. An increase in the prevalence of syphilis has been particularly noted in men who have sex with men (MSM) in places, such as the USA [17, 22-24].

3. Classification of *Treponema* species

Several aetiological agents responsible for diseases, such as syphilis (*Treponema pallidum* subsp. *pallidum*), Lyme disease (*Borrelia burgdorferi*) and leptospirosis (*Leptospira interrogans*) belong to the *Spirochaetaceae* family, as illustrated in Table 1 [25, 26]. The genus *Treponema* includes four treponemes that are of particular interest as human pathogens, namely: *T. pallidum* subsp. *pallidum* (syphilis), *T. pallidum* subsp. *pertenue* (yaws), *T. pallidum* subsp. *endemicum* (bejel) and *T. carateum* (pinta) [5, 25, 27]. *Treponema pallidum* subsp. *pallidum* is the only sexually transmitted pathogen [5]. There are several other treponemes, which include oral flora forms associated with periodontal disease, such as *Treponema denticola* and strains responsible for infections in animals, such as *T. paraluiscuniculi* (venereal spirochetosis in rabbits) and *T. brennaborense* [25, 27].

Table 1 Classification of *Treponema pallidum* subsp. *pallidum* [26].

Rank	Name
Kingdom	*Bacteria*
Phylum	*Spirochaetes*
Order	*Spirochaetales*
Family	*Spirochaetaceae*
Genus	*Treponema*
Species	*T. pallidum*
Subspecies	*pallidum*

The treponemes responsible for yaws, pinta and bejel are often collectively referred to as 'endemic' treponemes [5]. *Treponema pallidum* subsp. *pallidum* and these 'endemic' treponemes are morphologically indistinguishable, share a high level of DNA homology and are antigenically related [5, 12]. The main differences between these species are apparent in the geographical distribution, pathogenesis and host tissue specificity [5, 27]. Unlike in *T. pallidum* infections, endemic treponemes cannot lead to neurological complications or even be transmitted vertically (*in utero*) to the foetus [5]. It was not possible to distinguish between these subspecies until the discovery of molecular signatures, such as the flanking regions of the 15-kDa lipoprotein gene described in the study conducted by Centurion-Lara and colleagues [28].

4. Characteristics of *Treponema pallidum* subsp. *pallidum*

Treponema pallidum is a motile helical bacterium with a central protoplasmic cylinder, cytoplasmic membrane, peptidoglycan and outer membrane, which resembles a Gram-negative bacterium [5]. *Treponema pallidum* is typically thin, with six to 14 spirals and tapered ends [29]. The bacterium size ranges from a length of 6 μm to 20 μm and a width of 0.10 μm to 0.18 μm, which means light microscopy is inadequate for its visualisation, however, it can be viewed using dark-field microscopy[5, 20]. Some of the most characteristic features include the presence of periplasmic flagella and a paucity of immunogenic outer membrane spanning proteins, which have earned *T. pallidum* the name of "stealth pathogen" [5, 30]. Furthermore, *T. pallidum* is typically deprived of a few common features, such as a lipopolysaccharide (LPS) and an iron acquisition mechanism [5].

4.1. Culture characteristics

Characteristic features of *T. pallidum* include a slow generation time of 30 to 33 hours and an inability to grow *in vitro,* which may limit research but also implies that an antibiotic with a long half-life is required for treatment [5, 12, 30, 31]. Only a few generations of *T. pallidum* have been cultivated on rabbit epithelial cell monolayers when stored at 33°C to 35°C under micro-aerobic conditions [5, 20, 32]. Rabbit models have been used successfully for *in vivo* propagation of the spirochaete by inoculating the testis (rabbit infectivity test) [5, 31]. A primary infection and progression of disease similar to that in humans has been most closely portrayed in rabbit models compared to other animal models [5].

4.2. Genomic and metabolic characteristics

Treponema pallidum's genome was sequenced for the first time in 1998, at which time a small circular genome comprised of 1 138 006 bp and 1 041 open reading frames (ORF) was identified [7, 12, 20, 33]. The G+C content of the genome is 52%, which is higher than the 39% G+C content identified in *Borrelia burgdorferi*, a closely related species [7, 27]. No extra-chromosomal elements have been identified and the small size of the genome has led to speculation on whether this obligate pathogen relies on the biosynthetic pathways of the host and scavenging for compounds for survival [5, 7, 19]. Approximately 3% of the genome encodes lipoproteins, which are crucial constituents of the inner

membrane [20]. The immunogenic lipopeptides are hydrophilic and generally shielded from the antibodies induced [20].

The temperature-sensitive *T. pallidum* was noted to lack sigma-factor 32, which plays a crucial mediator role in the prokaryotic heat-shock response [33]. The treponeme's preference for low temperatures may account for the distinct distribution of the secondary syphilis lesions to the palms and soles of affected patients [6]. *Treponema pallidum*'s heat sensitivity elucidates why malaria therapy, a form of pyrotherapy, was successful in the treatment of syphilis in the past [6].

5. Virulence associated characteristics of *Treponema pallidum* subsp. *pallidum*

Attributes that contribute to the attachment, motility, invasion and immune-evasion capacity of *T. pallidum* play an important role in both the establishment and persistence of an infection in the host [8, 9]. Proteins or structures involved in each step of the pathogenic pathway can be associated with *T. pallidum*'s virulence [9]. *Treponema pallidum* is deprived of the typical virulence factors associated with other pathogenic bacteria, such as a capsule, LPS, potent exotoxins, an iron acquisition mechanism and an endotoxin in the outer membrane capable of inducing fever and inflammation [9]. The absence of these features is not a shortcoming in this instance as *T. pallidum* is not without its advantageous features, which include the presence of endoflaggella, treponemal proteins with fibronectin-binding properties, adhesins capable of binding to an extracellular matrix (ECM), production of lipoproteins capable of inducing expression of inflammatory mediators and the presence of several putative haemolysins of unknown function [6, 8, 12, 34]. *Treponema pallidum* has demonstrated antigenic variation in seven variable regions in the *T. pallidum* repeat (*tpr*) K gene, which could contribute to immune-evasion and establishing a chronic infection [3, 4, 6, 35]. The paucity of immunogenic outer membrane spanning proteins does not hamper its pathogenesis but aids it instead [5, 36].

5.1. Periplasmic flagella and motility

Motility is an important function associated with the haematogenous dissemination of pathogenic bacteria [6]. Corkscrew-like motility has been observed in *T. pallidum*, which is made possible by the presence of three to six periplasmic flagella attached at the subterminal ends of the cell [12, 25, 37, 38]. The classical flagellar appearance includes a basal body-motor complex, a hook and a filament [25, 37, 38].

The proteins involved in bacterial motility can be associated with the chemotaxis system or the basal body complex [12, 39]. A type III secretion system is believed to be necessary for auto-assembly of the flagella and once the apparatus is completed, a chemotaxic influence determines the direction of motility [40]. Only four genes have been recognised for their association in the chemotaxis system, which typically includes methyl-accepting chemotaxis transmembrane proteins (MCPs) and cytoplasmic chemotaxis (Che) proteins [7, 12]. The direction of movement can be guided by the former mentioned MCPs, which are capable of detecting attractants and repellents [12].

5.2. Outer membrane proteins

Initially, the first two proteins to be identified in *T. pallidum* were designated *Treponema pallidum* rare outer membrane protein 1 (Tromp 1) and *Treponema pallidum* rare outer membrane protein 2 (Tromp 2) [41, 42]. The former mentioned protein, Tromp 1, was found to share some amino acid sequence homology with streptococcal surface adhesins, thus alluding to a similar function [41]. The Tromp1 protein gene had been introduced into *E. coli* for expression and was subsequently shown to have porin activity and possible surface antigenic properties [41]. Specific surface antibody-binding properties were demonstrated by Tromp 2, a membrane spanning protein, when expressed in *E. coli* [42]. More outer membrane proteins have since been discovered and in some cases it has been found that the proteins do not necessarily traverse the bilipid layer, thus avoiding surface exposure [9]. One such example is protein Tp0453, which has been implicated in the increased permeability of the outer membrane by its localised destabilisation of outer membrane lipids [9]. The biological significance of such proteins could be to permit the entry of nutrients by increasing the permeability without sacrificing its characteristic paucity of surface antigens [9].

5.2.1. Proteins involved in attachment

An integral prerequisite in the initial invasion and dissemination process by the spirochaete is the ability to bind to host cell surface antigens, serum components, cell membranes and ECM components [6, 43, 44]. Extracellular matrix components that could be implicated in bacterial adherence include laminin, collagen I, hyaluronic acid, fibronectin, elastin and vitronectin [6, 43, 44]. In the case of binding to host cell receptors, of which integrins are suspected, a limitation in the number of spirochaetes could be considered a consequence of limited adhesion-ligand interactions [12].

5.2.2. Antigenic variation and immune evasion

Antigenic variation of surface proteins can be an invaluable contributor to the reinfection or persistence capabilities of a microorganism [45]. *Treponema pallidum* has one such recognised surface protein belonging to the *tpr* gene family

[45]. There are 12 paralogous genes belonging to the *tpr* gene family, which can be divided into three subfamilies [45]. Subfamily I includes genes *tpr*C, D, I and F; and subfamily II includes genes *tpr*E, G and J [45]. The subfamilies I and II encode products, which consist of conserved and unique regions but subfamily III differs in that variable regions may be encoded [45]. The *tpr*K of the latter subfamily genes was found to have seven variable regions of which some were proposed to have been created by gene conversion with donor regions adjacent to the *tpr*D gene [36, 45-47].

The heterogeneity of the *tpr*K gene within a strain of *T. pallidum* and the added evidence of stringent specificity of antibodies towards the *tpr*K gene regions is supportive of the idea that antigenic variation could accomplish immune evasion [47]. The major drive behind the evolution of the *tpr* gene has been intragenomic homologous recombination in *tpr* genes, which could in turn be influenced by adaptive immune responses [36, 45, 46, 47]. The gene conversion model proposed and conducted by Lafond *et al.* [3] in which *T. pallidum* Nichols strain was allowed to pass through naive rabbits for a period of 30 to 35 days, thus allowing adaptive immune responses to clear most of the treponemes, resulted in sequence variation in all seven variable (V) regions after only five passages, which is in line with the former proposed theory [3, 4, 36].

5.2.3. Lipoproteins activation of toll-like receptors

Treponema pallidum does not possess a true LPS, which would normally be responsible for immunological stimulation of the host cells but instead possesses lipid moieties of membrane associated proteins, which carry out the same function [33, 48, 49]. Lipoproteins can account for a large portion of the total protein composition in spirochaetes with as many as 22 putative lipoproteins predicted in *T. pallidum* [7, 50]. Examples of such lipoproteins in *T. pallidum* include the Tpp47, Tpp17 and GlpQ [50]. The lipoproteins are believed to be a principle membrane protein immunogen implicated in signal transduction by binding to the toll-like receptor 2, even though the component has not been confirmed to be situated on the outer surface membrane of *T. pallidum* [33, 50, 51]. The toll-like receptor 2 has a ten-fold greater sensitivity to lipoproteins than to Gram-negative bacteria's LPS [50]. A variety of cell types are activated *in vitro* by the presence of lipoproteins, such as monocytes, macrophages, lymphocytes and endothelial cells [50, 52, 53]. When the lipoproteins and lipopeptides bind with the CD-14 receptor in monocytes, it can induce both pro- and anti-inflammatory cytokines, such as: TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12 [33, 50, 54].

There are several mechanisms the spirochaetes use in an attempt to reduce surface exposure of protein antigens and the subsequent activation of the immune system [50]. The strategies used include hindering access of antibodies to the transmembrane outer-membrane proteins by some surface lipoproteins; by restricting lipoprotein to subsurface areas to avoid exposure and inducing antigenic variation of the surface lipoproteins [50]. Finally, the phosphatidyl glycerol derivative structure of the treponemal phospholipid was demonstrated to inhibit the induction of an immune response by blocking pathogen-associated molecular patterns (PAMPs) binding to cellular receptors and serum proteins [55]. Treponemal lipoproteins were shown to stimulate the CD14-dependent/ LBP-independent pathway and so an inhibitory function was achieved by blocking the CD14 function [55]. Ultimately, the inhibitory function of the phospholipids could aid in the persistence of treponemal infections [55].

6. Pathogenesis of *Treponema pallidum* subsp. *pallidum*

Syphilis is a multistage chronic illness, which is characterised by several stages of progression when untreated [5, 6, 56]. The progression of the illness is separated into the initial primary stage of infection, followed by the secondary, latent and tertiary stages of disease [5, 6, 57, 58]. Approximately 35% of the population with untreated latent syphilis is at risk of progressing into the tertiary stage of the disease, which is characterised by cardiovascular, neurological and other complications [6, 57].

Some of the greatest concerns with regard to *T. pallidum* infections include the altered progression of the disease in HIV infected persons to a more malignant accelerated form and the increased risk of HIV and syphilis co-infections [5, 6, 57]. Furthermore, there is a risk of congenital infections, which may result in a myriad of clinical manifestations if the child is born or result in the untimely death of the child [5, 57].

6.1. Routes of infection

The transmission of *T. pallidum* relies on intimate contact with the human host, which can occur during sexual intercourse, transplacentally to the foetus (congenital syphilis), during a blood transfusion or during an organ transplant [6, 8, 12, 57]. The latter two are rare routes of infection [6, 12]. The most common sexual route of transmission appears to be limited to the primary and secondary stages of disease and relies on contact with the lesions formed at these stages [12]. The estimated risk of infection is around 30% per sexual encounter [20, 59]. The required contact with lesions means there is a low risk of sexual transmission during the latent stage of disease despite the intermittent release of *T. pallidum* into the bloodstream during that period [5, 8, 12, 60]. The latent stage of disease may not present with any clinical manifestations but still poses a risk for congenital infection in untreated mothers as well as progression towards clinical diseases in immunecompromised patients [5].

6.2. Primary, secondary, latent and tertiary syphilis

Primary syphilis is typified by a painless chancre, which forms at the site of entry after an incubation period of approximately two to three weeks due to treponemal multiplication and the activation of immune responses that result in lymphocyte and macrophage infiltration [5, 6, 57]. Painless lesions are apparent when on the penis shaft, but can occur in inconspicuous sites, such as the anal canal, vagina, cervix or mouth, thus going undiagnosed in the primary stage of the disease [57, 58]. The lesions typically heal spontaneously, even when treatment has not been administered [12, 17, 58]. The main mechanism of clearance at this stage of the disease is believed to be accomplished by activated macrophage phagocytosis of opsonised *T. pallidum* [6, 12, 61].

The haematogenous spread of *T. pallidum* after the assumed invasion of intracellular junctions of endothelial cells in the primary stage of syphilis results in the progression of the disease into secondary syphilis [12, 17, 20]. Secondary syphilis is a systemic form of the disease characterised by several symptoms including: weight loss, sore throat, malaise, disseminated mucocutaneous rash, alopecia of scalp, local inflammation and generalised non-tender lymphadenopathy [5, 12, 17, 62]. The most notable clinical manifestation mentioned is the appearance of a widespread mucocutaneous rash, especially on the palms and soles [6, 57, 62]. The ulcers can also form what is described as "snail track" ulcers on the bucal mucosa and wart-like lesions in the genital region, referred to as condylomata lata [57, 58]. Approximately a quarter of secondary syphilis patients can experience relapsing episodes accompanied by recurrent rash, bucal ulcers and fevers [57, 58].

Secondary syphilis resolves spontaneously within three months and latent syphilis ensues [6, 12]. Early latent syphilis refers to the first year after infection, whilst late latent syphilis refers to an asymptomatic infection for more than a year [8, 12]. During that period, *T. pallidum* is released intermittently into the blood and the person may have a subclinical infection lasting up to a lifetime [8, 12]. Approximately a third of an untreated group of people go on to develop tertiary syphilis [6, 56-58]. In an era of effective antibiotic treatment *T. pallidum* complications present less frequently [5, 6, 59]. A waning immune system is suspected of being implicated in the progression of the disease into the tertiary phase [6, 57, 58]. The three main manifestations of tertiary syphilis include cardiosyphilis, gummas and neurosyphilis, which present in varying proportions in the affected population [58].

6.3. Neurosyphilis

Neurosyphilis is a neurological complication that results when *T. pallidum* penetrates the central nervous system (CNS) and is often identifiable by an increase in protein and leukocyte concentration in cerebrospinal fluid (CSF) or a reactive CSF-VDRL (Venereal Disease Research Lab) test [12, 17, 19, 63, 64]. Neurosyphilis used to be primarily associated with the tertiary stage of syphilitic infections but can in actual fact develop during the primary and secondary stages of the disease, thus rendering its classification as solely a complication of tertiary stage syphilis incorrect [19, 64, 65].

Neuroinvasion of *T. pallidum* is believed to follow one of three progressive routes [65]. Neuroinvasion can result in spontaneous resolution with no presentation of an inflammatory response or it might progress to transient meningitis, which resolves spontaneously [65]. Patients incapable of clearing *T. pallidum* may develop persistent meningitis, which is initially asymptomatic and can progress to a symptomatic disease form [65, 66]. Only 1% to 5% will develop a symptomatic CNS disease [66]. Typical clinical manifestations of neurosyphilis includes: tabes dorsalis, paresis, dementia, meningovascular syphilis, meningitis and amyotrophic lateral sclerosis [29, 57, 58, 65]. In the pre-antibiotic era the percentage of people admitted into asylums due to neurosyphilis ranged from 10% to 20% but it is no longer a common occurrence [66].

6.4. Congenital syphilis

In resource poor countries or in instances where prenatal screening is neglected, an untreated mother could pass the *T. pallidum* infection to her unborn child *in utero* or during delivery [20]. Congenital infections can result in abortion, stillbirth, neonatal mortality or severe sequelae [34].

The risk of vertical transmission can be as high as 95% during primary syphilis but rarely occurs during the tertiary stage of the disease [34, 67]. The consequence of vertical transmission during the primary and secondary stage of syphilis is pre-term birth or foetal death [20, 60]. In the event of the infected foetus' survival and birth, the child could demonstrate signs of growth impairments [5, 20, 60]. The pathogenesis of *T. pallidum* relies on the naturally suppressed immune response in the mother during pregnancy and the ability of the spirochaete to transverse the placenta [5]. Kasowit's law suggests that each successive pregnancy will have a greater chance of progressing normally due to less severe immune responses [67].

Postnatal congenital syphilis can be subdivided into late and early congenital syphilis depending on whether symptoms appear within the first two years of life or after two years [20]. Soon after birth the child may present with rhinitis, mucocutaneous lesions, osteochondritis of long bones, hepatosplenomegaly, lymphadenopathy, jaundice and neurosyphilis or may be asymptomatic [20, 34, 68]. In late congenital syphilis manifestations include: interstitial keratitis, neorosyphilis, Rhagades, sabre shins, sadle nose formation, eighth-nerve deafness and Hutchinson teeth [20, 34, 68].

7. Syphilis and HIV co-infection

Syphilis and HIV are both sexually transmissible diseases that have been recognised as important factors in each other's transmission and increase in incidence over the years [69, 70, 71]. Active, early syphilis may increase HIV transmission by two-fold or five-fold [72]. Human immunodeficiency virus positive individuals co-infected with a sexually transmitted disease (STD), such as syphilis, have demonstrated a higher viral load, which increases the risk of HIV transmission to a sexual partner [73, 74]. The disruption in the epithelium and mucosa shortly after *T. pallidum* infection can serve as an entry point for the HIV virus [18]. Another important observation is the local increase in CD4+ T-lymphocytes, which serves as a vehicle for a facilitated means of dissemination [18]. Dissemination in the body is further aided by the presence of lipoprotein in *T. pallidum,* which activates the expression of CCR5 on macrophages to facilitate viral entry [18, 75, 76, 77].

The infection of immunocompromised individuals might initially present with an atypical asymptomatic primary syphilis followed by a malignant form of secondary syphilis [78, 79]. Malignant syphilis can cause a myriad of conditions including wide-spread lesions with thick crusts, enlarged lymph nodes, myalgia and fever [79].

8. Antibiotic treatment of *Treponema pallidum* subsp. *pallidum*

Benzathine penicillin G is preferentially used for the treatment of syphilis and is the only treatment recommended by the Centres for Disease Control and Prevention (CDC) although second-line oral antibiotics, such as tetracyclines, macrolides and cephalosporins have been used alternatively [10, 11, 80, 81-83]. Benzathine penicillin G is administered intramuscularly (IM) as a single dose, which ideally eliminates the risk of re-infection due to noncompliance with dosage requirements while additionally providing a long lasting treponemicidal effect at 2.4 million units. Penicillin is currently the only treatment option available for infected pregnant women, who might require penicillin desensitisation prior to treatment in the case of known allergies to the antibiotic [11, 19, 83]. The treatment of neurosyphilis; however, requires an aqueous form of penicillin to successfully cross the blood-brain barrier [19].

Syphilis has been successfully treated with penicillin for over 50 years with no recorded resistance but the need for alternative treatment options for penicillin-sensitive patients and intolerance to intramuscular injections has spurred on the development of second-line antibiotics with similar efficacies, such as doxycycline, tetracycline and azithromycin [11, 19, 83-86]. The latter mentioned drug demonstrated efficacy comparable to the mainstay drug, penicillin, with just a single 2 g oral dose and has the added advantages of having good tissue penetration, long tissue life and few severe side effects [85, 87, 88]. The use of macrolides has not been without drawbacks particularly due to the rise in antibiotic resistance or problems resulting from reduced patient's compliance when multiple doses are required [17, 19, 84, 89]. A problem that may occur with any treatment during the secondary syphilis is that within the first 24 hours of treatment, the treponemal release of lipoproteins may result in an acute febrile condition known as the Jarisch-Herxheimer reaction [6]. The lack of LPS might explain the mild systemic response to the *T. pallidum* infections [6].

9. Mechanism of *Treponema pallidum* subsp. *pallidum* antibiotic resistance

The increase in occurrences of antimicrobial resistance is often associated with the prolonged use of an antimicrobial agent, which acts as a selective force [90, 91]. There are two mechanisms of resistance in bacteria, namely: innate resistance and acquired resistance [90, 91]. Acquisition of resistance can be as a result of *de novo* mutations or the acquisition of genetic material by susceptible bacteria through conjugation, transformation and transduction [90, 91, 92]. All the strains of a bacterial species with innate resistance are resistant to a particular antimicrobial [90, 91]. In contrast, acquired resistance presents a problem as susceptible strains of bacteria gain antimicrobial resistance and may proliferate under the selective pressure of the antimicrobial [90, 91].

9.1. Macrolide and tetracycline resistance

Unlike with penicillin; there has been an increase in *T. pallidum* resistance to macrolide antibiotics, especially erythromycin and azithromycin, which in turn has resulted in the increased use of tetracyclines for the treatment of syphilis [10, 11, 83, 84]. *Treponema pallidum*'s lack of resistance to penicillin treatment over the past five decades could be accounted for by the absence of plasmids, transposons and bacteriophages required for horizontal gene transfer (HGT), thus inhibiting the transfer of the penicillin resistance gene [83]. The absence of the elements implied that the resistance mutation could have been a result of spontaneous, endogenous chromosomal mutations [83].

*Treponema pallidum'*s resistance to macrolides has frequently been described to be a consequence of methylation or mutation in the peptidyl transferase region in domain V of the 23S rRNA of the 50S ribosomal subunit [10, 83]. A macrolide resistant strain, *T. pallidum* Street strain 14, was isolated from a penicillin-allergic syphilis patient in 1977 who was unresponsive to erythromycin treatment and upon further investigation was discovered to have an adenine (A) to guanine (G) transition at a position corresponding to the A2058G in the *E. coli* 23S rRNA gene [11, 83, 93, 94]. Another mutation in the 23S rRNA gene observed to confer resistance to spiramycin includes an A2059G mutation

discovered in clinical specimens from a Czech Republic patient, which is associated with resistance towards 14-, 15-, and 16- member lactone ring macrolides in other bacteria [83, 95]. The A2059G mutation has not been reported outside the Czech Republic [83]. A noteworthy observation is the effect of mutations at either the A2058 or A2059 in the corresponding peptidyl transferase centre in domain V of 23S rRNA in *E. coli*, which confer the maximum measurable level of resistance towards certain macrolides, whilst methylation of other regions, such as 2057, 2452 and 2611 only confer a low level antibiotic resistance due to its slightly removed location from the integral point for macrolide interaction [95].

The appearance of macrolide resistant *T. pallidum* was thought to be either due to a pre-existing resistant strain proliferating and spreading or a *de novo* mutation in response to antibiotic selective pressures [72]. The confirmation of macrolide resistance in multiple strains as opposed to one clonal strain is supportive of the idea that a mutation occurred [72]. Furthermore, individuals treated with macrolides within the prior year were found to be twice more likely to suffer an infection with a resistant strain [72]. Due to the rise in antibiotic resistance in *T. pallidum*, several authors are of the opinion that macrolides, such as azithromycin should not be administered in areas of high prevalence or to individuals with a high risk of acquisition of resistant strains [11, 72, 94].

The rise in macrolide resistance is believed to be aided by over-use of antibiotics for other sexually transmitted diseases (STDs) and non-sexually transmitted diseases, thus acting as a selective pressure in favour of resistant strains [83, 84, 96]. If caution is not practiced, the risk associated with the over-use of tetracycline is that it could follow the same fate as macrolides and be rendered ineffective in antimicrobial therapy of syphilis [83]. It has been hypothesised that a point mutation in the 16S rRNA gene of the 30S ribosomal unit could result in doxycycline resistance [83].

9.2. Intrinsic Resistance: Clindamycin and Rifampicin

In light of the wide-spread increase in resistance towards macrolide antibiotics in *T. pallidum*, surveillance of the efficacy of alternative antibiotics, such as tetracycline, is paramount. The rapid molecular detection of resistance in *T. pallidum* will thus be dependent on the understanding of underlying genetic mechanisms responsible [83]. Antibiotics toward which *T. pallidum* appear*s* intrinsically resistant to include clindamycin and rifampicin [83].

Similar to macrolide antibiotics, clindamycin has a binding site overlying the 23S rRNA region [83]. In a study conducted by Brause *et al*. [97] where the antibiotic efficacy was tested on rabbit models, it was observed that the reduction of treponemal cell counts was significantly lower for clindamycin with a five- to seven-fold decrease as compared to erythromycin and penicillin which brought about a 300-fold decrease [97]. Despite partial inhibition of protein synthesis having been recorded in a separate study by Stamm *et al.* [98] reports of clindamycin treatment failure was observed, including in pregnant women who subsequently gave birth to babies with congenital syphilis [98-101]. The genetic basis for the suspected intrinsic resistance in *T. pallidum* remains unknown but could be further exasperated in the presence of a mutation at the A2058G position in the *T. pallidum* Street strain 14; a mutation known to confer clindamycin resistance in other bacterial species, such as *Helicobacter pylori* [102, 103].

Intrinsic resistance to rifampicin is a trait shared by all spirochaetes, including *T. pallidum*. The binding site differs from clindamycin in that it binds the β-subunit of the DNA-dependent RNA polymerase (*rpo*B) [104, 105]. A mutation within the *rpo*B gene resulting in an amino acid substitution from asparagine (N) to serine (S), much like that in *Escherichia coli* S531, was theorised by Alekshun *et al.* [106] to be responsible for rifampicin resistance in spirochaetes, particularly *B. burgdorferi* [106]. The N531 substitution has been associated with intrinsic rifampicin resistance in *Mycobacterium celatum* and has been identified in several *Treponema* species, including *T. pallidum* Street strain 14 [105]. The evidence suggests that an N531 substitution is implicated in the intrinsic rifampicin resistance observed in *T. pallidum* [83].

10. Laboratory diagnosis of *Treponema pallidum* subsp. *pallidum*

The detection of *T. pallidum* can be broadly discussed under three topics, namely: microscopy, serology and molecular techniques. Due to the inability of the spirochaete to be cultured *in vitro* on routine bacteriological media or in rabbit epithelial cell monolayers for more than a few generations, detection has been mostly reliant on serological techniques and microscopy [94]. Microscopy is an immediate and direct technique employed for the detection of the small *T. pallidum* bacterium utilising dark-field microscopy or immunofluorescence in exudates from primary chancres and mucous membranes, which is seldom performed in the clinical setting and relies on the suspicion of the clinician [15, 60]. Microscopy is limited by the difficulty in detecting the spirochaete in less reliable mucous membrane lesion specimens, which may contain other indistinguishable saprophytic spirochaetes [15, 60].

The first serological test to be used for the detection of *T. pallidum* was introduced in 1906 by Wasserman but the lack in specificity led to the development of the current treponemal and non-treponemal serological tests [15, 60]. The latter-mentioned non-treponemal tests include Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) [15,60]. Although the non-treponemal tests are easy to perform, sensitive (93% to 98%) and quantifiable, false negatives due to the prozone phenomenon and false positives during acute viral infections, autoimmune disease, pregnancy, tuberculosis infections and hepatitis can occur [15,60]. The test is further limited by the inability to detect

primary syphilis in approximately 13% to 41% of individuals in the primary stage of the disease and a lower sensitivity for late stage syphilis of 60% to 75% [15, 60]. The traditional algorithm used for the screening of syphilis makes use of a non-treponemal assay typically followed by treponemal assays for confirmation [107]. Treponemal assays remain positive regardless of treatment and become reactive sooner thus successfully detecting infection in the primary stage earlier [15,60]. Examples of treponemal tests include: treponemal enzyme immunoassay (EIA), *T. pallidum* haemagglutination assay (TPHA), *T. pallidum* particle agglutination (TPPA), fluorescent treponemal antibody absorption test (FTA-abs), *T. pallidum* recombinant antigen line immunoassay and rapid point of care (POC) syphilis tests based on RPR and immunochromotographic based strips (ICT) [15]. The ICT has a sensitivity range of 93.7% to 100% and specificity ranging from 94.1% to 100% [15]. In an effort to reduce labour costs, several laboratories have begun to implement a reversed algorithm, especially in areas of low syphilis prevalence, by initial screening using the treponemal tests, which are usually based on an enzyme immunoassay (EIA) format [107]. The latter test has the advantages of being highly sensitive and specific, indicating true positive results, but cannot be solely relied apon and is confirmed using non-treponemal tests due to its inability to differentiate between past or concurrent infection [107]. The quantitative non-treponemal assays, such as RPR, also traditionally provide the added means of tracking treatment response by monitoring antibody titre change [107]. The pitfall of traditionally employed serological screening methods is its dependence on a functional immune response which presents a problem in immunecompromised populations, such as HIV positive people who are at the same time at a higher risk for acquisition and transmittance of sexually transmitted diseases [107].

Molecular methods are not commonly used in the detection of *T. pallidum* in a clinical setting but can be considered a complimentary technique to be used in combination with conventional dark-field microscopy or serology [5, 12, 13, 108]. Some of these molecular methods used for detection of the pathogen include the use of PCR and Real-time PCR assays [13, 14]. The application of PCR in the detection of *T. pallidum* DNA has the advantage of being a diagnostic method with the ability to characterise strains susceptible to macrolide antibiotics [94, 108]. The sensitivity of PCR detection assays has been found to vary depending on the specimen types and the stage of disease [94, 108].

11. Prevention, management and vaccine development

Numerous social factors can play a role in the incidence and spread of syphilis, which can be broadly discussed under the topics: populations of developing countries and the low socio-economic status of subgroups in a population [5, 17]. The risk factors associated with the spread of syphilis is poverty, drug use, limited education, multiple sexual partners, a positive HIV status, single status and a history of abortion and other sexually transmitted infections (STIs) [5, 20, 60, 109]. The risk of sexual transmission in HIV positive populations has partly been exacerbated by the increase in risky sexual behaviour, such as unprotected sex due to the optimism associated with the success of combination antiretroviral therapy [71, 77, 110]. Another contributing factor towards the increased incidence of syphilis in some developing countries is the lack of access or poor healthcare, which includes poor antenatal care [5, 60].

The prevention and management of *T. pallidum* infections is a multifaceted approach, which currently relies on the efficient detection and treatment of infected individuals, especially since no vaccine is available [57, 83]. The lack of a vaccine means that the preventative approach taken to reduce risk taking behaviour includes education of the population regarding the disease and the advertising of sexual protection [57]. Disease management relies on the identification of population groups at risk, efficient diagnosis of the disease and the treatment of the infected individuals, as well as any sexual contacts [82, 83]. Antenatal screening is of particular importance in the prevention of congenital syphilis [20, 57].

The development of a vaccine for syphilis has been unsuccessful despite many attempts over the years, which included rabbit model immunisation with whole-killed or attenuated *T. pallidum* as well as multiple intravenous doses of gamma-irradiated *T. pallidum* [19]. Though the latter mentioned study showed promise, the technique was both too impractical and expensive to pursue further investigation [19]. Other studies investigated the potential of immunisation with recombinant *T. pallidum* antigens with only partial protection resulting [19]. The development of a vaccine is further hampered by the genetic characteristic resulting in TprK antigenic variation [19].

12. Conclusion

Syphilis is a curable venereal disease, which despite a successful primary treatment, continues to affect people worldwide. A particular rise in incidence has been recorded in some countries and in population groups such as among men who have sex with men. The risk of the infection spreading and developing serious clinical manifestations has been further exacerbated due to the emergence of antibiotic resistant *T. pallidum* in clinical settings using second-line antibiotics preferentially. Studying this stealthy pathogen, known for host immune evasion, continues to present many challenges despite the progress made over the last few decades in understanding its pathogenesis within the human host. The devastating consequences of *T. pallidum* must be effectively monitored and prevented in the current absence of an

effective vaccine, particularly in light of the rising incidence of immunocompromised, HIV-infected individuals and common co-infection potential.

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