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Drug resistance and therapeutic advances in Treponema pallidum: A review

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Abstract. Treponema pallidum, a common pathogen in sexually transmitted diseases, has emerged as a significant public health threat in recent decades, showing a gradual increase in global prevalence. Antibiotics have been the primary method to treat Treponema pallidum infections. However, high-dose antibiotic use in clinical trials has raised concerns and unveiled a series of issues. This paper employs a literature review approach to summarize and compare scientific studies conducted in various countries and regions regarding Treponema pallidum in recent years. It aims to introduce and summarize the drug resistance of Treponema pallidum and the current progress in treatment. Studies indicate widespread resistance of Treponema pallidum to macrolides, while no resistance has been found to first-line drugs like benzathine penicillin and other alternative medications. Presently, benzathine penicillin remains pivotal in syphilis treatment. Alternatives such as ceftriaxone, second-generation tetracyclines, amoxicillin, and adjunctive immunomodulators, after clinical trials, have been used in early or late-stage syphilis treatment and prevention. The concept and research direction of syphilis vaccines are emerging and are poised to be a crucial approach in curtailing Treponema pallidum transmission. Diversified drug treatment strategies and the development of syphilis vaccines hold significant implications in safeguarding public health and ensuring global health security.

Keywords: Treponema pallidum, antibiotics, drug resistance, treatment

1. Introduction

Syphilis, caused by Treponema pallidum, is a prevalent sexually transmitted disease. It not only causes acute symptoms such as skin lesions, bone damage, and liver and spleen impairment but also leads to chronic damage including cardiovascular and joint abnormalities. The most severe clinical classification is neurosyphilis, which affects and damages the central nervous system. Congenital syphilis, transmitted from mother to child, severely endangers the health of the mother, fetus, and newborn, possibly resulting in miscarriage, premature birth, or stillbirth.

Antibiotics have long been proven effective in syphilis treatment. However, in recent years, the misuse of antibiotics and changes in human sexual behavior [1] have led to a global increase in drug-

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resistant strains of Treponema pallidum. This urgency necessitates the prevention and treatment of syphilis to reduce infection rates, morbidity, and mortality.

Following the establishment of benzathine penicillin as an essential medicine by the 69th World Health Assembly in May 2016, its widespread clinical use ensued. Benzathine penicillin, as the primary treatment for syphilis, effectively controls disease progression, and no clinical resistance cases have been reported, yet the drug has faced supply shortages in recent years. Presently, alternative drugs for syphilis treatment include macrolides and tetracyclines. While cases of treatment failure with macrolide antibiotics are not uncommon, resistant strains have emerged. Though tetracyclines have not exhibited resistance, their severe side effects impact patient efficacy [2]. Hence, active exploration and updating of alternative medications, along with the development of syphilis-related vaccines, intensified research on syphilis-related fundamentals, and global health encouragement, are urgently needed.

2. Overview of Treponema pallidum

2.1. Epidemiology of Treponema pallidum Infection

2.1.1. Overview of Epidemiology. Syphilis first erupted in Naples, Italy, during the 15th century. Due to the high infectivity of Treponema pallidum, this disease has become globally prevalent, emerging as one of the common sexually transmitted diseases. Typically, syphilis is prevalent in three countries with high incidence rates: the Solomon Islands, Equatorial Guinea, and Liberia. However, recent studies [3] show a global increase in syphilis cases from 8,845,220 in 1990 to 14,114,110 in 2019. This startling data indicates a roughly 60% rise in syphilis cases over the past 30 years.

2.1.2. Source of Infection. Humans are the sole source of Treponema pallidum, with both symptomatic and asymptomatic carriers playing crucial roles in its transmission. Treponema pallidum is present in the semen and blood components of infected individuals.

2.1.3. Modes of Transmission. Treponema pallidum primarily spreads through sexual intercourse, blood transmission, and mother-to-child transmission. Sexual contact remains the primary mode of syphilis transmission. This is largely due to engorgement and mucosal disruption during sexual activity, allowing the entry of Treponema pallidum from infected individuals to healthy individuals through bodily fluids.

Treponema pallidum can traverse the placental barrier at any stage of pregnancy, transmitted to the fetus via the umbilical vein. During childbirth, transmission can occur through direct contact with lesions. Additionally, neonates can be exposed to Treponema pallidum in breast milk, leading to infection.

Less common modes of transmission include indirect contact, where a few individuals living with patients unavoidably come into contact with contaminated household items like clothing and bedding, leading to infection. Medical procedures and kissing can also lead to syphilis transmission.

2.1.4. Susceptibility of Populations. Populations generally exhibit susceptibility to Treponema pallidum, and unprotected sexual contact significantly raises the incidence of the disease [4]. Changes in sexual behavior, especially among men having sex with men (MSM), pose a high risk for syphilis. In Germany, the syphilis incidence in men was 16 times higher than in women in 2015, with 85% of syphilis diagnoses among MSM [5]. Ignored changes in adolescent sexual behavior have also made them a high-risk group for syphilis. Reduced condom use among young people is a result of physiological and social influences. In the United States, between 2014 and 2016, there was a 24.5% increase in syphilis incidence among adolescents aged 14 to 19, a concerning statistic [1].

2.2. Immune Mechanisms of Treponema pallidum Infection

Following Treponema pallidum infection, the host experiences a robust immune response, primarily characterized by innate immunity and delayed hypersensitivity reactions. Cell-mediated immunity plays

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a crucial role, despite inducing complex antibody responses with relatively weak activity during the immune process.

2.2.1. Innate Immunity Against Treponema pallidum. Upon initial entry into the body, Treponema pallidum induces dermal fibroblasts to release interstitial collagenase (MMP-1) [6], reducing collagen levels in the dermis and impairing connective tissue's self-repair function, facilitating the invasion of Treponema pallidum. Macrophages are activated via Toll-like receptor (TLR) pathways [7]. In vitro experiments [8] have shown that Treponema pallidum induces M0 macrophages to polarize into M1 macrophages, increasing the secretion of interleukin IL-3β through NOD-like receptor family P1 (NLRP1), activating NLRP3 inflammasomes in macrophages, associated with the secretion of pro-inflammatory cytokine IL-1β. In the immune response against Treponema pallidum infection, early gamma-interferon (IFN-γ) plays a crucial role in Th1 immune responses, while Th1 cells mediate macrophage activation for Treponema pallidum phagocytosis [9]. Dendritic cells are also activated via TLR pathways, producing inflammatory mediators, including IL-6β, IL-12, IL-47, and TNF-α [10].

2.2.2. Cell-Mediated Immunity Against Treponema pallidum. Cell-mediated immunity against Treponema pallidum predominantly influences the immune process and is a common cause of clinical tissue damage. CD4+ and CD8+ T cells participate in and balance cellular immune responses in the host.

For primary syphilis patients, dendritic cells acquire phagocytosed antigens, activate T cells through antigen cross-presentation, and secrete cytokines like TNF and IFN-γ, exerting a potent anti-pathogen effect. CD4+ Th1 cells, when activated by IFN-γ, secrete inflammatory factors like IL-2, TNF-α, effective in clearing Treponema pallidum. Besides Th1, Th17, as a subtype of CD4+ cells, plays significant immunological roles by secreting IL-17 and promoting the secretion of chemokines like IL-8 [11]. Cytotoxic lymphocytes (CTL) exist in both primary and secondary syphilis lesions, with higher abundance of CD8+ T cells than CD4+ T cells during the same period [12]. After entering the second and third stages of syphilis, immune function becomes abnormal again. Interaction between Th1 and Th17 cells leads to decreased Th1 levels and increased Th2 levels. Th2 cells can secrete inflammatory mediators like IL-4, IL-10, dominating the later stages of syphilis immunity.

3. Resistance of Treponema pallidum

3.1. Resistance of Treponema pallidum to Macrolides

3.1.1. Mechanism of Treponema pallidum Resistance to Macrolides. Macrolide antibiotics held a crucial position in the clinical treatment of Treponema pallidum infections. These antibiotics primarily inhibit bacterial protein synthesis, irreversibly binding to the 50S subunit of bacterial ribosomes. Representative drugs include erythromycin and azithromycin.

With the gradual expansion of drug clinical use, macrolide resistance emerged during clinical treatments. In vitro experiments [13] demonstrated that resistance mechanisms of macrolide antibiotics often involve an adenine (A) to guanine (G) transition at position 2058 or 23 in the Escherichia coli 23S rRNA gene present in Street Strain14 strains. The A2058G mutation, absent in Nichols-type strains isolated until 1912, has been proven to be associated with macrolide antibiotic resistance. When a single base in the highly conserved main loop of the 23S rRNA structure domain V mutates, it alters the structure conformation, changing the antibiotic's target.

3.1.2. Resistance Situation of Treponema pallidum to Macrolides. Initially, erythromycin, derived from Streptomyces, was extracted to combat respiratory infections. Later, Keller and Morton in 1953 [14] first found erythromycin to be the most effective antibiotic against syphilis after penicillin. Subsequently, due to its significant therapeutic effect, erythromycin began widespread clinical use. However, in 1964 [15], a failed case of treating uterine syphilis with erythromycin occurred in a Houston hospital in the United States. Before this incident, it was established that erythromycin could pass through the placental

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barrier. In this case, a pregnant woman successfully treated syphilis two months before delivery, but unfortunately, signs of syphilis appeared in the newborn, who died on the third day after birth. In 1976, another case reported successful erythromycin treatment in the mother but inadequate treatment in the fetus [16]. Subsequent reports of erythromycin resistance emerged. Scientists began investigating these issues. Experimentally, Treponema pallidum strains (Street strain 14) isolated from a patient who failed erythromycin treatment exhibited high-level erythromycin resistance [13], where the 23S rRNA A2058G mutation affected the interaction between erythromycin and Treponema pallidum's target. To date, this resistant strain is prevalent in regions like the United States, Canada, and Xinjiang, China. Although the epidemiological impact of erythromycin on resistant strains hasn't been precisely reported, in 2021, the CDC removed erythromycin-related drugs from syphilis treatment regimens, indicating the complete abandonment of erythromycin treatment plans.

Azithromycin is a relatively newer macrolide antibiotic. In 1990, it was proven as effective as benzathine penicillin and erythromycin in treating active syphilis in rabbit models [17]. Subsequent studies in humans showed its efficacy in syphilis treatment in 2002 [18]. However, in San Francisco in the same year, the first treatment failure of syphilis with azithromycin was reported. Subsequently, resistant strains were isolated from patients who experienced treatment failure. Until 2015, the National South African treatment guidelines didn't include macrolide antibiotics for sexually transmitted diseases. However, in 2015, a 1g dose of azithromycin was confirmed as part of the treatment process. In the following years [19], epidemiological investigations found rapid emergence of azithromycin resistance and clinical treatment failures within areas of its use, significantly increasing the incidence of azithromycin-resistant strains in Treponema pallidum. In South Africa, azithromycin is no longer considered for syphilis treatment. Due to antibiotic misuse in certain regions of China, azithromycin is also not recommended for syphilis treatment.

For azithromycin-resistant strains, the first discovered mutation was the 23S A2058G mutation found in individuals who experienced treatment failure in San Francisco. However, this is not the only mutation. With an increased frequency of mutations during treatment, a second mutant sample, A2059G [20], was extracted from patients with treatment failure. Both mutant strains confer resistance to azithromycin treatment.

Despite the high mutation rate of the 23S rRNA against macrolides, further research on azithromycin gives hope for its reinstatement in syphilis treatment, especially in pregnant patients, in a relatively stable role. Therefore, monitoring mutations in Treponema pallidum and developing drugs are crucial for syphilis treatment.

3.2. Treponema pallidum Resistance to Tetracyclines

3.2.1. Microbial Mechanisms of Tetracycline Resistance. The antibacterial mechanism of tetracyclines involves specific binding to the 30S ribosomal subunit's A-site, inhibiting peptide elongation and bacterial protein synthesis. Tetracyclines have become common alternative drugs used in the treatment of Treponema pallidum infections.

Recent scientific research revealed that various bacteria develop resistance to tetracyclines primarily through gene mutations. For instance, Helicobacter pylori and Escherichia coli resistance to tetracyclines are associated with mutations at positions 965-968 (AGA) and 1058(G) in their 16S rRNA genes [21, 22]. The region at the 1058(G) site is crucial for peptide chain termination and accuracy of translation [23]. Moreover, point mutations at regions 926-928 and 939(A939C) in Helicobacter pylori's 16S rRNA gene have been proven to change the tetracycline binding sites, leading to resistance [24, 25].

Resistance might also relate to the genetic recombination of tet genes in bacterial genomes [26]. Several tet genes can integrate into mobile genetic elements like plasmids, transposons, and integrons, which, through bacterial reproduction, widely disseminate in the population, mediating efflux or ribosomal protectionn, consequently acquiring resistance [23]. Among these, the tet(M) gene is confirmed to play a major role in tetracycline resistance in organisms invading the urogenital tract (including Chlamydia, etc.) [27].

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Previous reviews distinctly indicate that the greater the antibiotic usage, the stronger the bacterial resistance to antibiotics, prompting strict restrictions or combination use of antibiotics worldwide. Brill et al. [28] demonstrated that individuals taking 100 mg of doxycycline daily increased the MIC of upper respiratory tract flora by 3.74 times; patients taking this doxycycline regimen were 5.77 times more likely to produce doxycycline-resistant strains compared to those taking a placebo. Other bacterial groups also developed resistance to high doses of tetracyclines, causing concerns among researchers about pathogen resistance in sexually transmitted diseases.

3.2.2. Treponema pallidum Resistance to Tetracyclines. Tetracyclines, as secondary recommended drugs for treating Treponema pallidum infections, have reported instances of tetracycline resistance [29]. However, based on current research results, there is no conclusive evidence demonstrating Treponema pallidum's resistance to tetracyclines.

Table 1. Recent Studies on Treponema pallidum Resistance to Tetracyclines in Different Regions

Researcher	Country/Region	Study Content	Research Result
Kou Caixia et al. [30]	China	Analysis of sample 16S rRNA gene resistance mutations and tetB gene amplification	No mutations related to tetracycline resistance were found at 16S rRNA gene sites; tetB gene amplification showed 8.3% positivity.
Fernández-Naval C et al. [31]	Barcelona	Amplification of specific regions of 16S rRNA and Sanger sequencing to search for mutations at 965 and 1058 sites	From 130 positive samples, a total of 108 (83.1%) 16S rRNA gene sequences were obtained, without mutations related to tetracycline resistance.
DE Souza RO et al. [32]	Brazil	Using BLASTn and RGI tools to detect antimicrobial resistance genes (groups) and protein mutations	Identified genes did not show resistance to tetracyclines used in syphilis treatment.
Sanchez A et al. [33]	France	Analysis of whether multiple point mutations exist in 16S rRNA genes	In patient samples studied, no mutations associated with tetracycline

Among Treponema pallidum strains, 14d/g and 14d/f are described as the two main strains globally. The predominant strain type in Europe is 14d/g [34-36], while in the Americas and Asia, it's 14d/f [35]. Although prevalent strains vary worldwide, the studies in Table 1 indicate that Treponema pallidum's 16S rRNA gene did not exhibit mutations related to tetracycline resistance. Noteworthy is the detection of tetB gene related to tetracycline resistance in T. pallidum-positive samples in China, suggesting this phenomenon might stem from extensive tetracycline usage in China and highlights the need for enhanced monitoring of Treponema pallidum's resistance to tetracyclines.

4. Treponema pallidum Infection Treatment Progress

4.1. First-Line Clinical Drug—Benzathine Penicillin

4.1.1. Summary of Benzathine Penicillin Application in Syphilis Treatment. Since the first clinical use of penicillin in 1941, research on its clinical applications has been ongoing. In the mid-20th century, the U.S. Public Health Service initiated large-scale efforts in syphilis prevention and treatment, and Kamp et al. [37] described their experience with benzathine penicillin G treatment for early syphilis. Over the decade following 1946, new cases of syphilis in the UK reduced by 95%, credited to the direct effects of penicillin treatment [38]. Interestingly, recent findings suggest penicillin's efficacy in treating syphilis

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in patients co-infected with HIV [39] and cases of acute proliferative glomerulonephritis secondary to syphilis [40].

As Treponema pallidum might require multiple mutations for resistance development against penicillin, resistance seems unlikely, with no evidence indicating such a risk [41]. High sensitivity of Treponema pallidum to benzathine penicillin has made it a frontline drug in syphilis clinical treatment. However, since benzathine penicillin was established in 2016 as the fundamental drug for treating syphilis infections, a global shortage has posed significant challenges to syphilis treatment [42].

4.1.2. Disadvantages and Adverse Reactions of Benzathine Penicillin Treatment. Despite its widespread use in syphilis treatment, benzathine penicillin has presented various issues in clinical use. Due to its unique structure, it cannot penetrate the blood-brain barrier, leading to poor efficacy in treating the crucial clinical symptom of neurosyphilis. Penicillin allergy remains the most common adverse reaction, but due to attention to such reactions, skin testing has become widespread, making these reactions less common today. Concerns about the safety of high-dose penicillin treatment have arisen. In a study in Shanghai, China [43], of 406 patients treated with penicillin for syphilis, 133 experienced adverse reactions, including liver and biliary system damage, hypokalemia, skin and appendage damage, worsening syphilitic symptoms, and neurological damage. Emphasizing the need for a well-optimized benzathine penicillin treatment regimen and close monitoring of patient conditions is crucial in syphilis treatment.

4.2. Alternative Treatment Drug—Ceftriaxone Sodium

Considering allergic reactions and other adverse effects following benzathine penicillin injections in some patients, research on alternative syphilis treatment drugs has gradually progressed. Ceftriaxone is a second-line drug for early syphilis treatment, and clinical use has shown that most patients allergic to penicillin can tolerate ceftriaxone. In vitro experiments in rabbit models, although not directly comparing penicillin and ceftriaxone for syphilis treatment, have demonstrated a minimum inhibitory concentration as low as 0.0006μg/ml [44] against Treponema pallidum. Some studies in Jiangsu, China, have also confirmed the effectiveness of ceftriaxone treatment in non-pregnant and immunocompetent early syphilis patients, comparable to benzathine penicillin treatment [45].

Interestingly, compared to penicillin, ceftriaxone has become an effective alternative treatment drug for early syphilis in HIV-1-infected individuals. Additionally, it can effectively treat asymptomatic neurosyphilis [46]. Neurosyphilis is a severe manifestation of syphilis that can cause irreversible damage to the central nervous system, leading to fatalities in severe cases.

Although there is still debate about the effectiveness of ceftriaxone in treating neurosyphilis, research has not halted. Evaluations of neurosyphilis treatment efficacy are based on identifying abnormalities in cerebrospinal fluid. Results from studies by C.M. Marra et al. [47] indicate no significant differences in serological and clinical efficacy between penicillin and ceftriaxone treatments for neurosyphilis in HIV-infected patients. However, due to insufficient experimental data, the exact clinical significance requires further investigation. Studies in France involving 208 patients assessing the effectiveness of both drugs in treating neurosyphilis similarly corroborated these findings [48]. Despite promising experimental results, direct clinical evidence is still lacking, necessitating extensive clinical trials to confirm the efficacy of ceftriaxone treatment.

4.3. Alternative Treatment Drug—Tetracycline

4.3.1. Summary of Tetracycline Application in Syphilis Infection Treatment. As resistance to macrolides in treating syphilis has increased worldwide, researchers have shifted focus to tetracycline-class drugs, exploring their feasibility as alternative treatments for syphilis infections.

Tetracycline, as a first-generation traditional tetracycline-class drug, induces adverse digestive system reactions such as stomach pain, diarrhea, nausea, and secondary infections, significantly impacting patient tolerance and compliance. Caution is warranted in the use of tetracycline-class drugs

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during pregnancy due to their teratogenic nature and the potential for yellow-brown tooth discoloration in children [2].

Doxycycline, a second-generation semi-synthetic tetracycline, compared to traditional tetracycline, exhibits a broader antibacterial spectrum, longer half-life, higher gastrointestinal absorption rate, [49] and stronger lipid solubility [50]. A review of pregnant women using doxycycline [51] suggested significant safety differences between doxycycline and tetracycline, indicating no correlation between using doxycycline during pregnancy and teratogenic effects or teeth discoloration in children, possibly due to doxycycline's weaker affinity for calcium. These more advanced pharmacokinetic characteristics and fewer adverse reactions have successfully substituted traditional tetracyclines in clinical use. A case study by Kory A. Tillery et al. [52] highlighted the potential safety and efficacy of doxycycline treatment before the 20th week of pregnancy for women allergic to penicillin who cannot undergo desensitization. However, further data analysis is required to validate this.

Researchers have evaluated the treatment effects of tetracycline-class drugs during different stages of syphilis infection, delineating the situation regarding their alternative treatment.

For early syphilis treatment, researchers generally consider doxycycline's treatment efficacy inferior to benzathine penicillin. A meta-analysis [53] on syphilis treatment suggested that ceftriaxone is more suitable as a penicillin alternative for early syphilis treatment compared to doxycycline. Clinical research by Jun Li et al. [54] reported lower serological treatment success rates for early syphilis than previously published (91.4% success rate for benzathine penicillin treatment compared to 82.6% for doxycycline).

In recent years, emerging minocycline, a second-generation semi-synthetic tetracycline antibiotic, demonstrated the strongest antibacterial effect, rapid oral absorption, fewer gastrointestinal side effects, and a longer half-life among similar antibiotics [55]. Therefore, many hospitals in China have adopted it as a treatment for syphilis. Clinical monitoring by Haoqing Wu et al. [55] revealed no statistically significant difference in efficacy between minocycline and benzathine penicillin for early syphilis treatment, especially showing superior efficacy for primary syphilis. However, current guidelines do not recommend minocycline treatment due to multiple influencing factors, necessitating more prospective studies on minocycline's early syphilis treatment.

For late-stage syphilis treatment, intravenous penicillin G for 10-14 days remains the gold standard [56] for treating neurosyphilis. The UK's national guidelines recommend doxycycline as an alternative treatment for neurosyphilis [57]. Case studies by R. Amode et al. [58] suggested that oral doxycycline could improve clinical management in patients diagnosed with ophthalmic syphilis (without evidence of neurosyphilis) while avoiding the inconvenience and difficulties associated with intravenous administration. Research by Nicolo` Giromett et al. [59] showed similar serological and clinical outcomes between 28 days of oral doxycycline treatment for early neurosyphilis and first-line parenteral treatment based on procaine penicillin G.

Doxycycline has shown some significant efficacy in late-stage syphilis treatment, possibly due to its high lipid solubility and high penetration through the blood-brain and blood-eye barriers. However, the evidence supporting this hypothesis is limited and based on measuring drug concentrations in cerebrospinal fluid and pharmacokinetic studies in small case series, [60] requiring further comprehensive analysis.

4.3.2. Tetracycline-Class Drug Use in Syphilis PrEP/PEP: A Brief Overview. Prophylactic administration of doxycycline has been considered a new public health strategy against the rising incidence of syphilis infections. Preventive medication is primarily divided into Pre-Exposure Prophylaxis (PrEP) and Post-Exposure Prophylaxis (PEP).

Presently recommended doxycycline PrEP and PEP [61] regimens involve taking 100mg of doxycycline monohydrate/hydrate daily during the exposure period or consuming 200mg within 72 hours after exposure (up to six tablets per week).

As early as 2011, a modeling study on the impact of doxycycline PrEP on syphilis infections among Australian MSM [62] estimated a potential reduction in syphilis infections by 50% after 12 months and

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85% after 10 years if 50% of MSM adhered to doxycycline PrEP with a 70% efficacy rate. While direct clinical evidence is lacking, this suggests a new direction for preventing the spread of syphilis infections.

Clinical research [63] on sexually active MSM indicated that doxycycline Post-Exposure Prophylaxis (PEP) might be effective, showing a roughly 70% reduced risk of syphilis infection among treated MSM compared to the control group. A recent doxycycline PEP trial [64] in the United States also demonstrated an 87% reduction in syphilis incidence among individuals taking HIV PrEP, providing strong evidence for doxycycline in preventing syphilis infections.

Although a survey [65] showed acceptability among most MSM for using doxycycline to prevent sexually transmitted infections like syphilis, concerns among clinical researchers and some public health organizations about its resistance and safety persist. This apprehension might stem from the emergence of tetracycline resistance in Neisseria gonorrhoeae, but currently, there's no evidence of tetracycline resistance in Treponema pallidum. While adults tolerate doxycycline well, it may lead to loss of human gut microbiota diversity and gastrointestinal disturbances, [66] necessitating extensive clinical practice to balance the beneficial effects of doxycycline in preventing syphilis infections and its adverse reactions.

4.4. Alternative Treatment Drug—Amoxicillin + Probenecid

Because Japan only obtained a supply of benzathine penicillin as late as 2022, the treatment guideline for Treponema pallidum infections [67] in Japan since 2002 has recommended daily administration of 1500mg of amoxicillin. However, recent studies have proposed a 3g/d amoxicillin combined with a probenecid regimen and compared it well with the guideline's 1.5g/d amoxicillin monotherapy.

Results from a study by Kazuhiko Ikeuchi et al. [68] in 2020 indicated that the 1.5g/day amoxicillin monotherapy for syphilis treatment is as effective as the standard therapy and may reduce accompanying adverse reactions during treatment. Pharmacokinetics show that daily oral intake of 1.5g amoxicillin can achieve sufficient serum Treponema pallidum killing levels.

A clinical study by Naokatsu Ando et al. [69] demonstrated that low-dose amoxicillin therapy and combined probenecid therapy had serological cure rates of 93.5% and 97.9%, respectively, for early syphilis. This study suggests that both regimens are comparable in efficacy to intramuscular benzathine penicillin G [70] and provide a basis for amoxicillin's ability to replace benzathine penicillin in treatment. Additionally, it further highlights slightly better efficacy with the combined probenecid therapy, showing a faster decrease in early syphilis RPR titers shortly after treatment. The occurrence of side effects between the two regimens showed no significant difference, making them potentially favorable options for treating syphilis infections.

As an alternative treatment drug, continuous intake of amoxicillin for over four weeks [71] poses a major drawback. Compared to intramuscular injection of benzathine penicillin and other alternative treatment drugs, prolonged medication not only leads to poor patient compliance but also, as an antibiotic of the penicillin class, can easily trigger Jarisch-Herxheimer reactions. These reactions manifest as fever, chills, diarrhea, rash, and other adverse effects caused by the secondary reaction to the massive death of Treponema pallidum, as corroborated in studies by YOUNG-MIN YANG et al [72].

4.5. Auxiliary Treatment Drug—Immunomodulators

In reference to the immune mechanisms against Treponema pallidum infections, in 2019, Wang Cuiyan et al. [73] focused on syphilis patients treated at hospitals over the past three years. They used ELISA to detect changes in IL-2 and IL-10 levels in different patients. Results revealed that syphilis patients had higher IL-2 and IL-10 levels in peripheral blood than the control group. However, as the disease progressed, IL-2 levels gradually decreased while IL-10 levels increased. This trend was more evident in patients with neurosyphilis excluded by syphilis serum fixation.

In the realm of immunotherapy, many Chinese researchers have proposed using immunomodulators to regulate the body's immune status, thereby assisting antibiotics in eliminating Treponema pallidum. Commonly used immunomodulators for clinical treatment include thymosin, recombinant human IL-2, and pidotimod.

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Thymosin is a bioactive peptide extracted from healthy calf thymus. It functions through Toll-like receptors in bone marrow and plasma-like dendritic cells, triggering the secretion of cytokines (such as IL-2, IFN-γ), promoting the activation and differentiation of dendritic cells and T cells, thereby enhancing immunity [74]. Unfortunately, in Yang Jinwu's study [75] on thymosin tablets combined with benzathine penicillin for early syphilis seroreversion, although the seroreversion rate increased, it lacked statistical significance. This might be due to factors like sample size, patient constitution, and medication compliance.

IL-2, secreted by activated T cells, promotes the proliferation and differentiation of lymphocytes. Recombinant human IL-2, as a novel immunomodulator, effectively prolongs IL-2 half-life in the body, enhances cellular immunity levels, and aids in clearing Treponema pallidum. This was corroborated in a study published in 2018 by Lu Jun et al. [76], yet further trials are required to ascertain the increase in RPR seroreversion rate.

Pidotimod is another synthetic dipeptide that promotes the production of cytokines like IL-2 and enhances phagocytosis and T cell proliferation. In a study by Li Weibin [77] in 2022 on benzathine penicillin combined with pidotimod for treating syphilis serum fixation, the combined treatment improved the $CD4^+$, $CD3^+$, and $CD4^+/CD8^+$ ratio, effectively shortened the resolution time of symptoms like rash and chancre. Feng Fan's study [78] also found that using ceftriaxone sodium combined with pidotimod improved the cure rate and increased peripheral blood NK cells, $CD3^+$, and $CD4^+$ cell levels, aligning with the former conclusion.

4.6. Research on Syphilis Vaccines

With extensive and continuous global use of the aforementioned antibiotics, drug resistance will inevitably pose a significant obstacle in the treatment process. Vaccines, recognized as important interventions in controlling disease transmission, have emerged as a promising avenue for syphilis research.

Miller [79] observed immune effects in rabbits 36 weeks after immunizing them with γ-ray-treated Nichols strain Treponema pallidum, suggesting sustained immunity for a year. Miller believed the outer membrane protein of Treponema pallidum was the primary factor inducing an immune response, laying the groundwork for studying outer membrane protein vaccines and demonstrating the feasibility of syphilis vaccination.

However, studies found that the outer membrane of Treponema pallidum lacks surface-exposed proteins [80], particularly phosphatidylcholine and lipopolysaccharides. Phosphatidylcholine [81], present in the cytoplasmic membrane [82], is a major lipid antigen targeted by antibodies in infected individuals [81]. Nonetheless, other components of the outer membrane, like glycolipids, do not exhibit immunoreactivity [81, 83]. These characteristics of outer membrane components [84] may contribute to its poor immunogenicity and pose challenges in developing outer membrane protein vaccines.

To address the low immunogenicity issue, researchers proposed using adjuvants and fusion antigens to enhance their immunogenicity and evoke better immune responses. IL-2, microparticles, and nanoparticles were considered. IL-2, mentioned earlier, can bolster immune responses, while nanoparticles, administered orally or intraperitoneally, allow direct transfection of dendritic cells and activation [85]. Scientists have successfully developed multi-valent fusion antigens like Gdp+TP0453 [86], demonstrating good immunogenicity. However, further research is needed to determine if they can provide complete immune protection.

Additionally, researchers discovered that Treponema pallidum can regulate the expression of several outer membrane proteins throughout its lifecycle [87]. PolyG repeat sequences in the Tp0126 protein [88] were found to modulate Tpr protein [89] expression at the transcriptional level in various spirochete strains. This high strain variation might facilitate immune evasion during Treponema pallidum infection, hindering the protective immunity of vaccines.

Compared to traditional vaccines, nucleic acid vaccines have emerged as a more flexible alternative. They have been successfully applied in preventing various animal diseases. Consequently, nucleic acid vaccines for syphilis have garnered recent research interest. However, genetic editing of Treponema

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pallidum is in its nascent stage, making it challenging to study the function of each gene product [90]. Moreover, due to Treponema pallidum's reproductive characteristics, how to mass culture and propagate it ex vivo remains a challenge in developing nucleic acid vaccines.

5. Discussion

Slowing down the resistance of Treponema pallidum has become a focal point in scientific research today. Reducing the long-term use and misuse of antibiotics, be it a specific one or a class of antibiotics, stands as a pivotal aspect in clinical diagnosis and treatment. Despite benzathine penicillin being a frontline therapeutic drug with good efficacy for early syphilis treatment, it remains in short supply in most countries globally. Moreover, some patients exhibit poor tolerance to it, leading to the emergence of various substitute drugs. However, the improper and excessive use of macrolides has resulted in widespread resistance of Treponema pallidum to them, emphasizing the need for vigilant monitoring of resistance to other drugs.

We believe that appropriately combining antibiotics in treatment will likely be a feasible approach in the future. This idea stems from Japanese scientists' application of amoxicillin combined with probenecid. For instance, combining benzathine penicillin with ceftriaxone sodium, through a rational dosage ratio, could mitigate the adverse reactions of penicillin. Simultaneously, leveraging ceftriaxone sodium's ability to penetrate the blood-brain barrier may result in the simultaneous eradication of Treponema pallidum in peripheral blood and cerebrospinal fluid, thereby achieving a favorable therapeutic effect. Since penicillins and tetracyclines exhibit antagonistic effects and tetracycline poses risks of resistance and potential multi-system adverse reactions, further research on the combined use of ceftriaxone sodium and tetracycline is imperative. It's important to adapt strategies case by case, accumulating experiences from multiple clinical treatments and trials to offer patients more suitable antibiotic therapies.

However, the effectiveness of antibiotics in preventing and treating syphilis will inevitably wane and eventually become obsolete, presenting an inevitable potential limitation. Therefore, the development of an effective syphilis vaccine should become a shared pursuit among dermatologists in the future.

Simultaneously, the immunomodulatory therapies researched in China provide robust evidence from an immunological perspective. Selecting the best immunomodulator to regulate patients' immune functions, assisting in the bactericidal action of antibiotics, accelerating the treatment pace, and improving treatment efficiency should be an integral part of subsequent clinical studies.

Preventing the spread of syphilis has also become a crucial measure for maintaining public health security. While this article mentions the use of tetracycline-class drugs for pre- and post-exposure prophylaxis, drug prevention alone may not be entirely effective and excessive reliance on medication may lead to a series of adverse reactions. Therefore, it's necessary to enhance education among male homosexual populations and women planning pregnancies, emphasizing strict avoidance of unsafe sexual practices, regular check-ups—especially for screening sexually transmitted diseases—and curbing the spread of syphilis and other sexually transmitted diseases at the source.

6. Conclusion

Through literature review and comparative analysis, this paper has provided a detailed introduction and elucidation of the epidemiology, immune mechanisms, resistance status, and treatment progress of syphilis.

Syphilis, as one of the common sexually transmitted diseases, exhibits a rapidly increasing incidence among young populations, especially in male homosexual communities. Research on frontline and alternative therapeutic drugs indicates that Treponema pallidum has developed widespread resistance to macrolides. Although no resistance has been found to other drugs used clinically, the use of high-dose antibiotics and the phenomenon of other pathogens (such as Neisseria gonorrhoeae) developing resistance to related drugs have sounded alarm bells for us.

Presently, benzathine penicillin remains crucial in syphilis treatment, while alternative drugs such as ceftriaxone sodium, amoxicillin in combination with probenecid for early syphilis treatment, and

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tetracycline-class drugs for syphilis prevention and late-stage treatment have shown promising results in clinical research. Additionally, immunomodulators like recombinant human IL-2 have been proven to assist in the bactericidal action of antibiotics, thereby enhancing treatment efficiency. However, the treatment effectiveness of alternative drugs still requires substantial clinical evidence, signifying a challenging path for the progress and update of treatment regimens.

Research on syphilis vaccines has commenced; however, exploration into the gene structure and function of Treponema pallidum remains in its infancy. Transitioning from traditional vaccines to target-specific nucleic acid vaccines involves creating suitable conditions for cultivating Treponema pallidum ex vivo, identifying conservative sequences in Treponema pallidum genes and proteomes through sequencing, or modifying easily mutable sequences and structures to enhance vaccine immunogenicity and stability. These issues pose challenges that must be overcome in the process of developing syphilis vaccines.

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