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MINIREVIEW

Global Challenge of Antibiotic-Resistant *Treponema pallidum*▽

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Syphilis is a multistage infectious disease that is usually transmitted through contact with active lesions of a sexual partner or from an infected pregnant woman to her fetus. Despite elimination efforts, syphilis remains endemic in many developing countries and has reemerged in several developed countries, including China, where a widespread epidemic recently occurred. In the absence of a vaccine, syphilis control is largely dependent upon identification of infected individuals and treatment of these individuals and their contacts with antibiotics. Although penicillin is still effective, clinically significant resistance to macrolides, a second-line alternative to penicillin, has emerged. Macrolide-resistant strains of *Treponema pallidum* are now prevalent in several developed countries. An understanding of the genetic basis of *T. pallidum* antibiotic resistance is essential to enable molecular surveillance. This review discusses the genetic basis of *T. pallidum* macrolide resistance and the potential of this spirochete to develop additional antibiotic resistance that could seriously compromise syphilis treatment and control.

Spirochetes are motile, spiral-shaped bacteria that are divided into the families *Spirochaetaceae*, *Brachyspiraceae*, and *Leptospiraceae* (54). *Treponema* species, which are members of the family *Spirochaetaceae*, are fastidious anaerobic or microaerophilic host-associated spirochetes. While the majority of *Treponema* species are found in the flora of humans and animals, a few species are pathogenic for humans. *Treponema pallidum* subspp. *pallidum*, *endemicum*, and *pertenue*, the agents of venereal syphilis, endemic syphilis, and yaws, respectively, together with *Treponema carateum*, the agent of pinta, are primary pathogens of humans that have eluded in vitro cultivation (70). *Treponema denticola* and certain other oral *Treponema* species that are associated with human periodontal disease are cultivable, opportunistic pathogens (22). The purpose of this minireview is to provide an overview of current antibiotic resistance in *T. pallidum* subsp. *pallidum* (*T. pallidum*), the most significant pathogen of the genus globally, and to discuss the potential of this spirochete to develop additional antibiotic resistance that could seriously compromise syphilis treatment and control.

EPIDEMIOLOGY OF SYPHILIS

Syphilis is a multistage disease that is usually transmitted through contact with active lesions of a sexual partner or from an infected pregnant woman to her fetus (70). Efforts to eliminate syphilis have met with only modest success (24). The World Health Organization (WHO) estimated that there were 12 million new cases of syphilis in 1999, with more than 90% of the cases occurring in developing countries (www.who.int/hiv/pub/sti/who_hiv_aids_2001.02.pdf). Congenital syphilis is a leading cause of stillbirth and perinatal mortality in many of these countries (66). Despite the availability of new diagnostic tests and antibiotic therapy, syphilis has reemerged in several developed countries. While the widespread epidemics of syphilis that occurred in Russia in the 1990s and more recently in China mostly involved heterosexuals, smaller outbreaks in the United States, Canada, and England predominately involved men who have sex with men (MSM) (5, 10, 43, 67, 77). However, recent increases in syphilis rates for U.S. women and infants suggest that heterosexually transmitted syphilis may be an emerging problem in the United States (5). A major concern associated with increased rates of syphilis is that active, early syphilis (i.e., primary and secondary stages) enhances transmission of human immunodeficiency virus (HIV) by 2- to 5-fold, thus promoting the spread of HIV (14, 70).

ANTIBIOTIC TREATMENT AND RESISTANCE

Effective antibiotic treatment is a key component of syphilis control programs (4). According to the U.S. Centers for Disease Control and Prevention (CDC) 2006 guidelines, the recommended treatment for uncomplicated, early syphilis in adults is penicillin G benzathine administered intramuscularly (i.m.) as a single dose of 2.4 million units (MU) (4). This form of the drug provides weeks of treponemicidal levels of penicillin in the blood, though it does not efficiently cross the blood-brain barrier (39, 48; for further details on the form and dose of penicillin for treatment of syphilis, see reference 4.) Because there are no proven alternatives to penicillin for treatment of infected pregnant women, those who are penicillin allergic should be desensitized and then treated with penicillin G benzathine. Despite over 65 years of extensive clinical experience with penicillin, the need to administer this antibiotic parenterally has led to the use of second-line oral antibiotics, including macrolides (e.g., erythromycin and azithromycin) and tetracy-

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clines (e.g., tetracycline and doxycycline), as first-line drugs for treatment of syphilis. This use of alternative antibiotics, which is inconsistent with current CDC guidelines, occurs more frequently outside the United States (76). The resistance of *T. pallidum* to macrolides as well as two additional classes of antibiotics is discussed below.

Macrolide resistance. Macrolides are bacteriostatic antibiotics that inhibit protein synthesis by binding reversibly to 23S rRNA of the 50S ribosomal subunit (80). Shortly after introduction of erythromycin, the first macrolide, in the 1950s, resistance to this antibiotic was observed in several bacterial pathogens (61). Failure of erythromycin treatment for syphilis was reported by South et al. (69) in 1964 and Fenton and Light (15) in 1976 in pregnant women who delivered infants with congenital syphilis. However, since erythromycin may not efficiently cross the placental barrier, it is unknown if these treatment failures were actually due to erythromycin-resistant *T. pallidum* (55). In 1977, *T. pallidum* Street strain 14 was isolated from a U.S. patient with active lesions of secondary syphilis who failed long-term erythromycin therapy (74, 75). Studies by Stamm et al. (73, 74), using an in vitro assay to assess the effect of antibiotics on treponemal protein synthesis, showed that Street strain 14 is resistant to high levels of erythromycin and cross resistant to azithromycin, a newer macrolide that was approved by the U.S. Food and Drug Administration (FDA) in the early 1990s. In vivo studies with the rabbit model of syphilis confirmed that Street strain 14 is resistant to erythromycin and azithromycin (37). Interestingly, the macrolide-resistant phenotype of Street strain 14 is highly stable, despite multiple passages in laboratory rabbits in the absence of antibiotic pressure (41; L. V. Stamm, unpublished data).

Macrolide resistance is often associated with alteration of the target site (i.e., the peptidyl transferase region in domain V of 23S rRNA) via mutation or methylation (61, 80). For over two decades after its isolation, the genetic basis of Street strain 14 macrolide resistance remained a mystery. However, the complete sequence of the 1.14-Mb genome of the *T. pallidum* Nichols strain, published in 1998, provided some helpful clues (17). Analysis of the genome sequence revealed that *T. pallidum* lacks genetic elements (e.g., plasmids, bacteriophage, and transposons) commonly associated with horizontal gene transfer mechanisms (i.e., transformation, transduction, and conjugation) that are a major means for acquiring antibiotic resistance (60, 61). Based on this observation, it seemed plausible that Street strain 14 macrolide resistance originated endogenously via a spontaneous, low-frequency, chromosomal mutation in the 23S rRNA gene that conferred a survival advantage to treponemes exposed to macrolides. Consistent with this hypothesis, Stamm and Bergen (71) demonstrated that an adenine (A)-to-guanine (G) transition, at the position cognate to A2058 in the *Escherichia coli* 23S rRNA gene, is present in both copies of the Street strain 14 23S rRNA gene (Table 1) (7). This mutation was not present in the *T. pallidum* Nichols strain, the type strain, which was isolated in 1912 and is sensitive to macrolides. Sequencing of the Street strain 14 genome to high accuracy with oligonucleotide arrays recently confirmed the A-to-G transition in both 23S rRNA genes (45). Point mutations at position A2058 have been identified in many other macrolide-resistant bacteria, including *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*, agents of swine dysentery and spirochetal diarrhea, respectively (Table 1) (28, 29, 56, 61, 65, 80). Additionally, Lee et al. (35) reported that an A2058G mutation, present in one or both copies of the *T. denticola* 23S rRNA gene, conferred equivalent, high-level resistance to erythromycin (Table 1). Macrolide-resistant *T. denticola* isolates were obtained after in vitro exposure to erythromycin, suggesting that antibiotic pressure is sufficient to select mutants with point mutations that confer high-level resistance.

TABLE 1. 23S rRNA gene mutations associated with spirochete macrolide resistance

Spirochete	Mutation position[a]	Resistance(s)[b]	Clinical/induced[c]	Reference[d]
T. pallidum	A2058G	Ery, Azi	Clinical	71
	A2059G	Spi	Clinical	44
T. denticola	A2058G	Ery	Induced	35
B. hyodysenteriae	A2058G	Ery, Cli, Tyl	Induced	28
	A2058T	Ery, Cli, Tyl	Clinical	28
B. pilosicoli	A2058G	Tyl	Induced	56
	A2058T	Ery, Cli, Tyl	Clinical	29
	A2059C	Ery, Cli, Tyl	Clinical	29
	A2059G	Ery, Cli, Tyl	Clinical	29
Brachyspira spp.	A2062C	Tyl	Induced	56

[a] Based on the numbering of the *E. coli* 23S rRNA gene nucleotide sequence.
[b] Based on the antibiotics tested. Azi, azithromycin; Cli, clindamycin; Ery, erythromycin; Spi, spiramycin; Tyl, tylosin.
[c] Macrolide resistance present in clinical/field isolate versus experimentally induced resistance.
[d] Initial description of mutation.

In hindsight, it is remarkable that the first documented report of clinically relevant macrolide resistance in *T. pallidum* Street strain 14 was not greeted with greater apprehension (21, 75). Indeed, after the introduction of azithromycin, there was considerable enthusiasm for the use of this macrolide for treatment of syphilis, since unlike erythromycin, it can be administered orally as a single 2-g dose and has a long tissue half-life (30). The clinical efficacy of azithromycin for treatment of syphilis was demonstrated in nonrandomized studies (30, 31, 79) and randomized controlled trials in the United States (23, 26), Africa (59), and China (2) that compared the cure rates for azithromycin and penicillin. Azithromycin was used for syphilis treatment in Uganda in the mid-1990s (31), in the United States (San Francisco, CA) in 1999 and 2000 (47), and in the United States (Los Angeles, CA) and Canada (Vancouver, BC) in 2000 (9, 58). However, treatment failures were observed in eight patients in San Francisco from 2002 to 2003 (6, 30). Molecular analysis of *T. pallidum* in clinical specimens from two of these patients revealed the presence of a 23S rRNA gene mutation identical to the Street strain 14 A2058G mutation (37). Retrospective analysis of clinical specimens from Baltimore, San Francisco, Seattle, and Vancouver showed that while the prevalence of the A2058G mutation varied among these sites, it increased significantly over time within the sites that were analyzed temporally (30, 37, 50). For example, the percentage of San Francisco clinical specimens containing *T. pallidum* with the A2058G mutation increased from 4% in 2002 to 41% in 2003, 56% in 2004, and 76.5% in 2005 (30, 47).

The percentage of Seattle clinical specimens containing *T. pallidum* with the A2058G mutation increased from 9% in 2002 to 23% in 2003, 50% in 2004, and 56% in 2005 (41). Although only a limited number of clinical specimens were analyzed in these studies, the rapidly increasing frequency of detection of the A2058G mutation is nevertheless disturbing.

Macrolide-resistant *T. pallidum* with the A2058G mutation is now present in several areas of the United States, Canada, Europe, and China (37, 42, 43, 44, 47, 50). Although some outbreaks are due to multiple macrolide-resistant strains that emerged independently, there is also evidence for the limited spread of a macrolide-resistant clone of *T. pallidum* (41, 42). The findings of Marra et al. (41) support the hypothesis that recent macrolide use for unrelated infections (e.g., oral, skin, respiratory, and genital infections) contributes to the increased prevalence of macrolide-resistant *T. pallidum* by providing a selective pressure. Interestingly, macrolide-resistant *T. pallidum* appears to be uncommon in some developing countries (e.g., Madagascar, Tanzania, and Uganda) (30, 31, 59). However, because macrolide use is increasing in developing countries, it is likely that macrolide-resistant *T. pallidum* will eventually emerge or will be introduced via travel and/or tourism. Although potentially problematic in resource-limited settings, availability of a recently developed real-time PCR assay for detection of the A2058G mutation and, with modification, a new mutation (A2059G; see below) would enable molecular surveillance efforts for rapid identification of macrolide-resistant *T. pallidum* in populations where macrolide use has been limited and therapy might still be effective (53). Regardless of the setting, the use of macrolides for treatment of syphilis must be undertaken with the utmost caution, particularly in areas where the prevalence of macrolide resistance is unknown. All syphilis patients who receive macrolides must be closely monitored both clinically and serologically. Clinical specimens (i.e., lesion exudate, blood, and cerebrospinal fluid [CSF]) from patients who fail treatment should be subjected to molecular analysis for detection of mutations in the *T. pallidum* 23S rRNA genes. Monitoring for resistance is important not only to identify individual treatment failures but also to more efficiently target control programs that are based on the prevalence and geographical distribution of resistance.

Until recently, the A2058G mutation was the only documented mutation associated with macrolide resistance in *T. pallidum*. In vitro studies by Stamm et al. (73, 74) suggested that this mutation confers resistance to 14-member (e.g., erythromycin and roxithromycin) and 15-member (e.g., azithromycin) lactone ring macrolides but not to 16-member lactone ring macrolides (e.g., spiramycin, midecamycin, and tylosin), possibly due to the differential binding of these antibiotics to the 23S rRNA of Street strain 14. In 2009, Matejkova et al. (44) reported the identification of a new mutation (i.e., A2059G) in the 23S rRNA gene of *T. pallidum* in clinical specimens from a Czech Republic patient with secondary syphilis who did not respond to spiramycin therapy. The A2059G mutation is associated with resistance to 14-, 15-, and 16-member lactone ring macrolides in a number of bacteria, including *B. pilosicoli*, and is likely responsible for spiramycin resistance in *T. pallidum* (Table 1) (29, 80). The prevalence of the A2059G or the A2058G mutation among clinical specimens collected from syphilis patients in the Czech Republic is ~18% for either mutation (44). There was no direct epidemiological relationship among syphilis patients with the A2059G mutation, suggesting that these individuals had not been infected with a single macrolide-resistant strain. Currently, there are no reports outside of the Czech Republic of infections due to *T. pallidum* harboring the A2059G mutation. However, recent experience with the A2058G mutation suggests that emergence of *T. pallidum* with the A2059G mutation is unlikely to be unique to a specific geographical region, particularly if this mutation extends the spectrum of macrolide resistance to include resistance to erythromycin and azithromycin (80).

Clindamycin resistance. Clindamycin, a semisynthetic derivative of lincomycin, was introduced in the 1960s for treatment of bacterial infections (13). Although chemically unrelated to the macrolides, clindamycin is grouped with these antibiotics since its binding site on 23S rRNA overlaps with those of macrolides. Clindamycin resistance can result from modification of the 23S rRNA target site by mutation, methylation, or through efflux or inactivation of the antibiotic (33). In 1976, Brause et al. (3) reported the use of a rabbit model of intradermal infection to determine the relative efficacies of clindamycin, erythromycin, and penicillin for treatment of infection with the *T. pallidum* Nichols strain. Single i.m. injection of two different doses of clindamycin (15 mg/kg and 40 mg/kg) did not significantly affect treponemal cell counts. Multiple i.m. injections of clindamycin reduced treponemal cell counts by 5- to 7-fold, whereas multiple i.m. doses of erythromycin and penicillin reduced treponemal cell counts by >300-fold, suggesting that *T. pallidum* has a level of intrinsic resistance to clindamycin. In vitro studies by Stamm et al. (74) with *T. pallidum* Nichols strain and Street strain 14 showed that while clindamycin partially inhibited protein synthesis in both strains, the effect on the Nichols strain was somewhat stronger. Failure of clindamycin treatment to cure syphilis in humans was reported by Meljanac et al. in 1999 (46) and by Woznicova in 2007 (84). In the latter report, an infected pregnant woman treated with clindamycin, which has been shown to cross the placenta (55), gave birth to an infant with congenital syphilis. DNA sequences of *T. pallidum* present in the infant's lesions matched those of Street strain 14. Based on limited clinical data, it appears that clindamycin is unlikely to be effective for treatment of syphilis, presumably due to the intrinsic resistance of *T. pallidum* to clindamycin. However, it should be noted that the A2058G mutation present in *T. pallidum* Street strain 14 is associated with high-level clindamycin resistance in some isolates of *Helicobacter pylori*, *B. hyodysenteriae*, and certain other bacterial species (Table 1) (28, 29, 81). Thus, the higher level of clindamycin resistance observed by Stamm et al. (74) for Street strain 14 may be due to an additive effect of the A2058G mutation on the intrinsic level of clindamycin resistance in this spirochete. The genetic basis of the latter is unknown.

Rifampin resistance. Rifampin binds to the β-subunit of DNA-dependent RNA polymerase (RpoB) encoded by the *rpoB* gene, thus preventing RNA synthesis. All spirochetes tested thus far, including *T. pallidum*, are intrinsically resistant to rifampin (36, 72). Several investigators have reported the absence of a treponemicidal effect of rifampin on humans with syphilis (27). Rifampin has been used as a selective agent for the isolation of cultivable *Treponema* from human or animal specimens in which bacterial contaminates are present (e.g.,

oral cavity, genital and intestinal tracts, and bovine foot and mammary lesions, etc.). If continuous in vitro cultivation of *T. pallidum* is eventually achieved, rifampin resistance, though not clinically relevant, would enable the use of this antibiotic for culturing *T. pallidum* from syphilis patients, thus benefiting molecular and epidemiologic studies.

Rifampin resistance is usually due to mutations in the *rpoB* gene that lead to changes in the RpoB amino acid sequence, resulting in the reduced binding of rifampin by RpoB. Alekshun et al. (1) proposed that rifampin resistance in *Borrelia burgdorferi*, and possibly other spirochetes, is due to the substitution of an asparagine (N) for a serine (S) at the RpoB residue cognate to *E. coli* S531. Lee et al. (34) observed a N531 substitution in the RpoB amino acid sequence of 22 *Borrelia* strains. Stamm et al. (72) reported that the N531 substitution is present in *T. pallidum* Street strain 14 and in several other *Treponema* species, including *T. denticola*. Analysis of the *T. pallidum* Nichols strain genome sequence confirmed the presence of the N531 substitution in this spirochete. While not commonly observed in rifampin-resistant bacteria, the N531 substitution is associated with high-level resistance in *Mycobacterium celatum*, an organism that is intrinsically resistant to rifampin (32). Thus, the N531 substitution in RpoB is likely responsible for the intrinsic resistance of *T. pallidum*, as well as other spirochetes, to rifampin.

POTENTIAL MECHANISMS FOR DEVELOPMENT OF RESISTANCE TO TETRACYCLINES AND PENICILLIN

In view of the emergence of clinically significant macrolide resistance in *T. pallidum*, it is of paramount importance to consider how this spirochete could develop resistance to tetracycline, an alternative antibiotic, and to penicillin, the recommended first-line antibiotic for syphilis treatment. If resistance to these drugs emerges, awareness of potential mechanisms would enable a more focused approach to detecting mutations in *T. pallidum* that are known to be associated with tetracycline or penicillin resistance in other bacteria. Identification of the genetic basis of resistance is a prerequisite for developing molecular methods (e.g., real-time PCR assays and oligonucleotide arrays) for the rapid detection and surveillance of antibiotic-resistant *T. pallidum* in clinical specimens. Presumably, the latter information will also aid clinicians in the choice of antibiotic for syphilis treatment.

Tetracycline resistance. Tetracyclines are bacteriostatic antibiotics that inhibit protein synthesis by binding reversibly to 16S rRNA of the 30S ribosomal subunit (60). Four small-scale studies demonstrated that doxycycline, a tetracycline derivative with better bioavailability, with a more convenient dosing schedule, and with fewer gastrointestinal side effects than tetracycline, has a success rate similar to that of penicillin for treatment of early adult syphilis (18, 20, 52, 83). No treatment failures were reported for three of the four studies. However, one patient in the study by Onoda (52) did not show serological evidence of response to treatment at 4 months after doxycycline therapy. Since this patient was lost to follow-up, it is unclear as to why the patient was nonresponsive. However, treatment failure due to doxycycline resistance is a possibility. Tetracycline resistance, which confers cross-resistance to doxycycline, can be due to efflux systems, ribosomal protection proteins, mutation of certain ribosomal proteins, or enzymatic inactivation of the antibiotic (60). Additionally, single point mutations in the 16S rRNA genes of *H. pylori* and *Propionibacterium* spp. at residues cognate to positions 965 to 967 or 1058, respectively, in the *E. coli* 16S rRNA gene confer tetracycline or doxycycline resistance in these bacteria (64, 85). Pringle et al. (57) hypothesized that point mutations in the 16S rRNA genes of spirochetes, which have one or two copies of this gene, could result in decreased susceptibility to doxycycline, as observed with *B. hyodysenteriae* (i.e., G1058C). Based on this information and knowledge of *T. pallidum* genetics, it appears that point mutations in one or both copies of the 16S rRNA gene (7) are the most plausible mechanism for development of doxycycline resistance. Due to macrolide resistance, doxycycline may become more widely used as an alternative drug for syphilis. Since treatment requires a 14-day course of 100 mg of doxycycline taken orally twice daily, compliance is an issue (4). If treatment failure occurs in patients who have been compliant, the 16S rRNA genes of *T. pallidum* in the patients' clinical specimens should be high-priority targets for detection of mutations associated with doxycycline resistance.

Penicillin resistance. Penicillins and cephalosporins are bactericidal β-lactam antibiotics that interfere with the action of transpeptidase enzymes (i.e., penicillin binding proteins [PBPs]) that carry out the cross-linking of the cell wall of actively growing bacteria (86). Penicillin is the only antibiotic currently recommended by the CDC for treatment of all stages of syphilis (4). The form of penicillin (i.e., benzathine, aqueous procaine, or aqueous crystalline), dose, and duration of treatment are dependent upon the stage and clinical manifestations of syphilis (4). Although reports of penicillin treatment failures, particularly for patients with HIV infection, are not uncommon, to date there is no documented penicillin resistance in *T. pallidum* (14, 16, 62, 75). Most serologically defined treatment failures are thought to be due to reinfection or to patient-to-patient variation in the decline of nontreponemal test titers after treatment (i.e., ≥4-fold decrease), rather than to relapse, which is rare. However, it can be difficult to distinguish between reinfection and relapse since molecular methods for epidemiologic typing of *T. pallidum* may lack discriminatory power (51).

It is important to note that *T. pallidum* can invade the central nervous system (CNS) early in infection and that some strains may have a greater propensity for neuroinvasion (38, 39). Rolfs et al. (62) demonstrated that *T. pallidum* was present before therapy in at least one-fourth of patients with early syphilis, regardless of their HIV infection status. This finding is similar to those of other studies (38). Nonetheless, most patients with early, uncomplicated syphilis respond well to treatment with penicillin G benzathine i.m., although this therapy does not result in treponemicidal levels of the antibiotic in cerebrospinal fluid (CSF) (48, 62). However, because *T. pallidum* has been isolated from CSF following i.m. administration of 2.4 to 10.8 MU of penicillin G benzathine, the CDC recommends that patients with known CNS involvement be treated with 18 to 24 MU/day of aqueous crystalline penicillin G administered at 3 to 4 MU intravenously (i.v.) every 4 h or by continuous infusion for 10 to 14 days (4, 38, 78).

Myint et al. (51) proposed that treponemes that survive in the CNS are responsible for the clinical relapse that occurs

when therapeutic levels of antibiotic wane in body fluids and tissues. This appears to be the case, since a more intensive course of high-dose aqueous penicillin given i.v. can cure relapse (16, 19, 78). Such information provides a basis for speculating how *T. pallidum* might develop penicillin resistance. Presumably, treponemes that invade the CNS of patients with early syphilis would encounter subtherapeutic levels of antibiotic that act as a selective pressure for mutants with low-level penicillin resistance when the patients are treated with i.m. penicillin G benzathine. This resistance would not be readily detected since it would be easily overcome when the patients are retreated with higher doses of i.v. penicillin upon treatment failure. Continued passage of treponemes with low-level penicillin resistance to new hosts, prior to treatment of relapsing infection, and repeated exposure of these treponemes to increasing levels of penicillin might eventually select mutants with a clinically significant level of penicillin resistance.

Unlike macrolide or tetracycline resistance, for which a single point mutation can confer stable, high-level resistance, penicillin resistance often involves the acquisition of new genetic information via horizontal gene transfer (86). The latter is unlikely to occur in *T. pallidum* due to the lack of plasmids, bacteriophage, or transposons (17). Strategies commonly used by bacteria to resist the effect of penicillin include production of β-lactamases that inactivate penicillin, acquisition of novel PBPs with low affinity for penicillin, alterations of PBPs through homologous recombination, changes in the structure and number of porins resulting in decreased permeability to penicillin, efflux pumps that decrease the intracellular concentration of penicillin, or various combinations of these strategies (86). Analysis of the *T. pallidum* genome sequence predicts three putative PBPs but no typical β-lactamases (17). However, Cha et al. (8) showed that Tp47, an abundant, membrane-bound lipoprotein that was initially identified and characterized by Norgard and colleagues (12, 82), binds penicillin and has high β-lactamase activity that is subject to strong product inhibition. Furthermore, Cha et al. (8) hypothesized that if a mutant variant of Tp47 emerges that overcomes the product inhibition of its β-lactamase activity, this would confer novel, bona fide resistance to penicillin. Fortunately, the Tp47 β-lactamase does not appear to be active against cephalosporins, leaving available the option of using these drugs for syphilis treatment should a Tp47 mutant emerge (25, 40, 49, 68, 87). It is important to note that the absence of documented penicillin resistance in *T. pallidum* after more than 6 decades of its use for treatment of syphilis suggests that the development of penicillin resistance will likely require a multistep mutational process whose probability of occurrence is much rarer than those of the single point mutations that are responsible for macrolide resistance. Although this may have forestalled the emergence of penicillin-resistant *T. pallidum*, it provides no guarantee that such resistance will not emerge.

CONCLUSION

Syphilis has many of the hallmarks of a disease that should be susceptible to elimination and perhaps ultimately to eradication, since (i) infected humans are the only natural reservoir; (ii) diagnostic methods, though not perfect, are relatively cheap and widely available; and (iii) early infection is usually treatable with a single dose of penicillin G benzathine (63). However, the recent, widespread syphilis epidemic in China, where syphilis had been virtually eliminated, and the resurgence of syphilis in many Western countries emphasize the urgent need for renewed vigilance (10, 11). In the absence of a vaccine, syphilis control is largely dependent upon identification of infected individuals and treatment of these individuals and their contacts with antibiotics. Although penicillin treatment is still effective, clinically significant resistance to macrolides has emerged in *T. pallidum* and is prevalent in several countries, including China. Macrolide resistance has compromised the effectiveness of azithromycin for syphilis treatment, such that patients who receive this antibiotic must be closely monitored for treatment failure. This development clearly warrants placing tighter restrictions on the use of azithromycin, a drug that was once deemed the most promising alternative to penicillin for syphilis treatment, particularly in settings where injections are problematic or for nonpregnant, penicillin-allergic patients (21, 30).

To date, there is no documented resistance of *T. pallidum* to the tetracyclines, which are the other main class of alternative antibiotic used for treatment of early syphilis in adults (4). Decreased use of macrolides could result in increased use of tetracyclines. Inadequate tetracycline therapy due to poor patient compliance could provide selective pressure for resistant mutants. If tetracycline-resistant *T. pallidum* were to emerge and spread, it would undoubtedly have an effect on syphilis control, particularly if the prevalence and geographical distribution of macrolide-resistant *T. pallidum* increase. This problem would be exacerbated if macrolide-resistant *T. pallidum* were also to develop resistance to the tetracyclines, since there are few alternatives to penicillin, except for ceftriaxone, which has not been extensively tested in clinical settings (25, 40, 49, 68, 87).

The global persistence of syphilis and the emergence and rapid spread of macrolide-resistant *T. pallidum* are reminders that there is no room for complacency. Addressing these issues requires renewed effort on several fronts, including the following: (i) developing new, single-dose oral antibiotics to ensure patient compliance; (ii) improving availability and reliability of rapid diagnostic tests to identify those who need treatment; (iii) supporting molecular surveillance to enable early detection of antibiotic-resistant *T. pallidum*; and (iv) pursuing development of a vaccine to prevent infection and thereby limit the need for antibiotics. We have been warned that the time is at hand when antibiotics will no longer be useful for the treatment of many infectious diseases due to the emergence and spread of multidrug-resistant bacteria. We must not allow this to come to pass for syphilis, the disease once designated by U.S. Surgeon General Thomas Parran as the "shadow on the land" (14).

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REFERENCES

1. **Alekshun, M., M. Kashlev, and I. Schwartz.** 1997. Molecular cloning and characterization of *Borrelia burgdorferi rpoB*. Gene **186:**227–235.
2. **Bai, Z. G., K. H. Yang, Y. L. Liu, J. H. Tian, B. Ma, D. H. Mi, L. Jiang, J. Y. Tan, and Q. Y. Gai.** 2008. Azithromycin vs. benzathine penicillin G for early

syphilis: a meta-analysis of randomized clinical trials. Int. J. STD AIDS **19:**217–221.
3. **Brause, B. D., J. S. Borges, and R. B. Roberts.** 1976. Relative efficacy of clindamycin, erythromycin, and penicillin in treatment of *Treponema pallidum* in skin syphilomas of rabbits. J. Infect. Dis. **134:**93–96.
4. **Centers for Disease Control and Prevention, K. A. Workowski, and S. M. Berman.** 2006. Sexually transmitted diseases treatment guidelines, 2006. MMWR Recommend. Rep. **55**(RR-11)**:**1–94.
5. **Centers for Disease Control and Prevention.** December 2008. Sexually transmitted diseases surveillance, 2007. U.S. Department Health and Human Services, Atlanta, GA.
6. **Centers for Disease Control and Prevention.** 2004. Brief report: azithromycin treatment failures in syphilis infections—San Francisco, California, 2002–2003. MMWR Morb. Wkly. Rep. **53:**197–198.
7. **Centurion-Lara, A., C. Castro, W. C. van Voorhis, and S. A. Lukehart.** 1996. Two 16S-23S ribosomal DNA intergenic regions in different *Treponema pallidum* subspecies contain tRNA genes. FEMS Microbiol. Lett. **143:**235–240.
8. **Cha, J. Y., A. Ishiwata, and S. Mobashery.** 2004. A novel beta-lactamase activity from a penicillin-binding protein of *Treponema pallidum* and why syphilis is still treatable with penicillin. J. Biol. Chem. **279:**14917–14921.
9. **Chen, J. L., D. B. Callahan, and P. R. Kerndt.** 2002. Syphilis control among incarcerated men who have sex with men: public health response to an outbreak. Am. J. Public Health **92:**1473–1474.
10. **Chen, Z. Q., G. C. Zhang, X. D. Gong, C. Lin, X. Gao, G. J. Liang, X. L. Yue, X. S. Chen, and M. S. Cohen.** 2007. Syphilis in China: results of a national surveillance programme. Lancet **369:**132–138.
11. **Cohen, M. S., G. E. Henderson, P. Aiello, and H. Zheng.** 1996. Successful eradication of sexually transmitted diseases in the People's Republic of China: implications for the 21st century. J. Infect. Dis. **174**(Suppl. 2)**:**S223–S229.
12. **Deka, R. K., M. Machius, M. V. Norgard, and D. R. Tomchick.** 2002. Crystal structure of the 47-kDa lipoprotein of *Treponema pallidum* reveals a novel penicillin-binding protein. J. Biol. Chem. **277:**41857–41864.
13. **Dhawan, V. K., and H. Thadepalli.** 1982. Clindamycin: a review of fifteen years of experience. Rev. Infect. Dis. **4:**1133–1147.
14. **Douglas, J. M., Jr.** 2009. Penicillin treatment of syphilis-clearing away the shadow on the land. JAMA **301:**769–771.
15. **Fenton, L. J., and I. J. Light.** 1976. Congenital syphilis after maternal treatment with erythromycin. Obstet. Gynecol. **47:**492–494.
16. **Fowler, V. G., Jr., G. L. Maxwell, S. A. Myers, C. R. Shea, C. N. Livengood III, V. G. Prieto, and C. B. Hicks.** 2001. Failure of benzathine penicillin in a case of seronegative secondary syphilis in a patient with acquired immunodeficiency syndrome: case report and review of the literature. Arch. Dermatol. **137:**1374–1376.
17. **Fraser, C. M., S. J. Norris, G. M. Weinstock, O. White, G. G. Sutton, R. Dodson, M. Gwinn, E. K. Hickey, R. Clayton, K. A. Ketchum, E. Sodergren, J. M. Hardham, M. P. McLeod, S. Salzberg, J. Peterson, H. Khalak, D. Richardson, J. K. Howell, M. Chidambaram, T. Utterback, L. McDonald, P. Artiach, C. Bowman, M. D. Cotton, C. Fujii, S. Garland, B. Hatch, K. Horst, K. Roberts, M. Sandusky, J. Weidman, H. O. Smith, and J. C. Venter.** 1998. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. Science **281:**375–388.
18. **Ghanem, K. G., E. J. Erbelding, W. W. Cheng, and A. M. Rompalo.** 2006. Doxycycline compared with benzathine penicillin for treatment of early syphilis. Clin. Infect. Dis. **42:**e45–e49.
19. **Greene, B. M., N. R. Miller, and T. E. Bynum.** 1980. Failure of penicillin G benzathine in the treatment of neurosyphilis. Arch. Intern. Med. **140:**1117–1118.
20. **Harshan, V., and W. Jayskumar.** 1982. Doxycycline in early syphilis: a long term follow up. Indian J. Dermatol. **27:**119–124.
21. **Holmes, K. K.** 2005. Azithromycin versus penicillin G benzathine for early syphilis. N. Engl. J. Med. **353:**1291–1293.
22. **Holt, S. C., and J. L. Ebersole.** 2006. The oral spirochetes: their ecology and role in the pathogenesis of periodontal disease, p. 323–356. *In* J. D. Radolf and S. A. Lukehart (ed.), Pathogenic *Treponema* molecular and cellular biology. Caister Academic Press, Norfolk, England.
23. **Hook, E. W., III, D. H. Martin, J. Stephens, B. S. Smith, and K. Smith.** 2002. A randomized, comparative pilot study of azithromycin versus benzathine penicillin G for treatment of early syphilis. Sex. Transm. Dis. **29:**486–490.
24. **Hook, E. W., III, and R. W. Peeling.** 2004. Syphilis control—a continuing challenge. N. Engl. J. Med. **351:**122–124.
25. **Hook, E. W., III, R. E. Roddy, and H. H. Handsfield.** 1988. Ceftriaxone therapy for incubating and early syphilis. J. Infect. Dis. **158:**881–884.
26. **Hook, E. W., III, J. Stephens, and D. M. Ennis.** 1999. Azithromycin compared with penicillin G benzathine for treatment of incubating syphilis. Ann. Intern. Med. **131:**434–437.
27. **Huigen, E., and E. Stolz.** 1974. Action of rifampicin on *Treponema pallidum*. Br. J. Vener. Dis. **50:**465.
28. **Karlsson, M., C. Fellstrom, M. U. Heldtander, K. E. Johansson, and A. Franklin.** 1999. Genetic basis of macrolide and lincosamide resistance in *Brachyspira* (*Serpulina*) *hyodysenteriae*. FEMS Microbiol. Lett. **172:**255–260.
29. **Karlsson, M., C. Fellstrom, K. E. Johansson, and A. Franklin.** 2004. Antimicrobial resistance in *Brachyspira pilosicoli*, with special reference to point mutations in 23S rRNA gene associated with macrolide and lincosamide resistance. Microb. Drug Resist. **10:**204–208.
30. **Katz, K. A., and J. D. Klausner.** 2008. Azithromycin resistance in *Treponema pallidum*. Curr. Opin. Infect. Dis. **21:**83–91.
31. **Kiddugavu, M. G., N. Kiwanuka, M. J. Wawer, D. Serwadda, N. K. Sewankambo, F. Wabwire-Mangen, F. Makumbi, X. Li, S. J. Reynolds, T. C. Quinn, the Rakai Study Group, and R. H. Gray.** 2005. Effectiveness of syphilis treatment using azithromycin and/or benzathine penicillin in Rakai, Uganda. Sex. Transm. Dis. **32:**1–6.
32. **Kim, B. J., S. H. Lee, M. A. Lyu, S. J. Kim, G. H. Bai, S. J. Kim, G. T. Chae, E. C. Kim, C. Y. Cha, and Y. H. Kook.** 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). J. Clin. Microbiol. **37:**1714–1720.
33. **Leclercq, R.** 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. **34:**482–492.
34. **Lee, S. H., B. J. Kim, J. H. Kim, K. H. Park, S. J. Kim, and Y. H. Kook.** 2000. Differentiation of *Borrelia burgdorferi* sensu lato on the basis of RNA polymerase gene (*rpoB*) sequences. J. Clin. Microbiol. **38:**2557–2562.
35. **Lee, S. Y., Y. Ning, and J. C. Fenno.** 2002. 23S rRNA point mutation associated with erythromycin resistance in *Treponema denticola*. FEMS Microbiol. Lett. **207:**39–42.
36. **Leschine, S. B., and E. Canale-Parola.** 1986. Rifampin resistant RNA polymerase in spirochetes. FEMS Microbiol. Lett. **35:**199–204.
37. **Lukehart, S. A., C. Gordones, B. J. Molini, P. Sonnett, S. Hopkins, F. Mulcahy, J. Engelman, S. J. Mitchell, A. M. Rompalo, C. M. Marra, and J. D. Klausner.** 2004. Macrolide resistance in *Treponema pallidum* in the United States and Ireland. N. Engl. J. Med. **351:**154–158.
38. **Lukehart, S. A., E. W. Hook III, S. A. Baker-Zander, A. C. Collier, C. W. Critchlow, and H. H. Handsfield.** 1988. Invasion of the central nervous system by *Treponema pallidum*: implications for diagnosis and treatment. Ann. Intern. Med. **109:**855–862.
39. **Marra, C. M.** 2009. Update on neurosyphilis. Curr. Infect. Dis. Rep. **11:**127–134.
40. **Marra, C. M., P. Boutin, J. C. McArthur, S. Hurwitz, G. Simpson, P. A. J. Haslett, C. van der Horst, T. Nevin, E. W. Hook III, and the AIDS Clinical Trials Group (ACTG).** 2000. A pilot study evaluating ceftriaxone and penicillin G as treatment agents for neurosyphilis in human immunodeficiency virus-infected individuals. Clin. Infect. Dis. **30:**540–544.
41. **Marra, C. M., A. P. Colina, C. Godornes, L. C. Tantalo, M. Puray, A. Centurion-Lara, and S. A. Lukehart.** 2006. Antibiotic selection may contribute to increases in macrolide-resistant *Treponema pallidum*. J. Infect. Dis. **194:**1771–1773.
42. **Martin, I. E., W. Gu, Y. Yang, and R. S. Tsang.** 2009. Macrolide resistance and molecular types of *Treponema pallidum* causing primary syphilis in Shanghai, China. Clin. Infect. Dis. **49:**515–521.
43. **Martin, I. E., R. S. Tang, K. Sutherland, P. Tilley, R. Read, B. Anderson, C. Roy, and A. E. Singh.** 2009. Molecular characterization of syphilis in patients in Canada: azithromycin resistance and detection of *Treponema pallidum* DNA in whole-blood samples versus ulcerative swabs. J. Clin. Microbiol. **47:**1668–1673.
44. **Matejkova, P., M. Flasarova, H. Zakoucka, M. Borek, S. Kremenova, P. Arenberger, V. Woznicova, G. M. Weinstock, and D. Smajs.** 2009. Macrolide treatment failure in a case of secondary syphilis: a novel A2059G mutation in 23S rRNA gene of *Treponema pallidum* subsp. *pallidum*. J. Med. Microbiol. **58:**832–836.
45. **Matejkova, P., M. Strouhal, D. Smajs, S. J. Norris, T. Palzkill, J. F. Petrosino, E. Sodergren, J. E. Norton, J. Singh, T. A. Richmond, M. N. Molla, T. J. Albert, and G. M. Weinstock.** 2008. Complete genome sequence of *Treponema pallidum ssp. pallidum* strain SS14 determined with oligonucleotide arrays. BMC Microbiol. **8:**76.
46. **Meljanac, N., E. Dippel, and C. C. Zouboulis.** 1999. Superimposed primary chancre in a patient with Adamantiades-Behcet's disease. Sex. Transm. Infect. **75:**124–125.
47. **Mitchell, S. J., J. Engelman, C. K. Kent, S. A. Lukehart, C. Gordones, and J. D. Klausner.** 2006. Azithromycin-resistant syphilis infection: San Francisco, California, 2000–2004. Clin. Infect. Dis. **42:**337–345.
48. **Mohr, J. A., W. Griffths, R. Jackson, H. Saadah, P. Bird, and J. Riddle.** 1976. Neurosyphilis and penicillin levels in cerebrospinal fluid. JAMA **236:**2208–2209.
49. **Moorthy, T. T., C. T. Lee, K. B. Kim, and T. Tan.** 1987. Ceftriaxone for treatment of primary syphilis in men: a preliminary study. Sex. Transm. Dis. **14:**116–118.
50. **Morshed, M. G., and H. D. Jones.** 2006. *Treponema pallidum* macrolide resistance in BC. CMAJ **174:**349.
51. **Myint, M., H. Bashiri, R. D. Harrington, and C. M. Marra.** 2004. Relapse of secondary syphilis after benzathine penicillin G. Molecular analysis. Sex. Transm. Dis. **31:**196–199.
52. **Onoda, Y.** 1979. Therapeutic effect of oral doxycycline on syphilis. Br. J. Vener. Dis. **55:**110–115.

53. **Pandori, M. W., C. Gordones, L. Castro, J. Engelman, M. Siedner, S. Lukehart, and J. Klausner.** 2007. Detection of azithromycin resistance in *Treponema pallidum* by real-time PCR. Antimicrob. Agents Chemother. **51:**3425–3430.
54. **Paster, B. J., and F. E. Dewhirst.** 2006. The phylogenetic diversity of the genus *Treponema*, p. 9–18. *In* J. D. Radolf and S. A. Lukehart (ed.), Pathogenic *Treponema* molecular and cellular biology. Caister Academic Press, Norfolk, England.
55. **Philipson, A., L. D. Sabath, and D. Charles.** 1973. Transplacental passage of erythromycin and clindamycin. N. Engl. J. Med. **288:**1219–1221.
56. **Prapasarakul, N., K. Ochi, and Y. Adachi.** 2003. In vitro susceptibility and a new point mutation associated with tylosin-resistance in Japanese canine intestinal spirochetes. J. Vet. Sci. **65:**1275–1280.
57. **Pringle, M., C. Fellstrom, and K. E. Johansson.** 2007. Decreased susceptibility to doxycycline associated with a 16S rRNA gene mutation in *Brachyspira hyodysenteriae*. Vet. Microbiol. **123:**245–248.
58. **Rekart, M. L., D. M. Patrick, B. Chakraborty, J. J. L. Maginley, H. D. Jones, C. D. Bajdik, B. Pourbohloul, and R. C. Brunham.** 2003. Target mass treatment for syphilis with oral azithromycin. Lancet **361:**313–314.
59. **Riedner, G., M. Rusizoka, J. Todd, L. Maboko, M. Hoelscher, D. Mmbando, E. Samky, E. Lyamuya, D. Mabey, H. Grosskurth, and R. Hayes.** 2005. Single-dose erythromycin versus penicillin G benzathine for the treatment of early syphilis. N. Engl. J. Med. **353:**1236–1244.
60. **Roberts, M. C.** 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol. Lett. **245:**195–203.
61. **Roberts, M. C.** 2008. Update on macrolide-lincosamide-streptogramin, ketolides, and oxazolidinone resistance genes. FEMS Microbiol. Lett. **282:** 147–159.
62. **Rolfs, R. T., M. R. Joesoef, E. F. Hendershot, A. M. Rompalo, M. H. Augenbraun, M. Chiu, G. Bolan, S. C. Johnson, P. French, E. Steen, J. D. Radolf, and S. Larsen.** 1997. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The Syphilis and HIV Study Group. N. Engl. J. Med. **337:**307–314.
63. **Rompalo, A. M.** 2001. Can syphilis be eradicated from the world? Curr. Opin. Infect. Dis. **14:**41–44.
64. **Ross, J. I., E. A. Eady, J. H. Cove, and W. J. Cunliffe.** 1998. 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. Antimicrob. Agents Chemother. **42:**1702–1705.
65. **Ross, J. I., E. A. Eady, J. H. Cove, C. E. Jones, A. H. Ratyal, Y. W. Miller, S. Vyakrnam, and W. J. Cunliffe.** 1997. Clinical resistance to erythromycin and clindamycin in cutaneous propionibacteria isolated from acne patients is associated with mutations in 23S rRNA. Antimicrob. Agents Chemother. **41:**1162–1165.
66. **Schmid, G. P., B. P. Stoner, S. Hawkes, and N. Broutet.** 2007. The need and plan for global elimination of congenital syphilis. Sex. Transm. Dis. **34**(Suppl.)**:**S5–S10.
67. **Simms, I., K. A. Fenton, M. Ashton, K. M. Turner, E. E. Crawley-Boevey, R. Gorton, D. R. Thomas, A. Lynch, A. Winter, M. J. Fisher, L. Lighton, H. C. Maguire, and M. Solomou.** 2005. The re-emergence of syphilis in the United Kingdom: the new epidemic phases. Sex. Transm. Dis. **32:**220–226.
68. **Smith, N. H., D. M. Musher, D. B. Huang, P. S. Rodriguez, M. E. Dowell, W. Ace, and A. C. White, Jr.** 2004. Response of HIV-infected patients with asymptomatic syphilis to intensive intramuscular therapy with ceftriaxone or procaine penicillin. Int. J. STD AIDS **15:**328–332.
69. **South, M. A., D. H. Short, and J. M. Knox.** 1964. Failure of erythromycin estolate therapy in in utero syphilis. JAMA **190:**70–71.
70. **Stamm, L. V.** 2001. *Treponema pallidum*, p. 1795–1808. *In* M. Sussman (ed.), Molecular medical microbiology, 1st ed. Academic Press, London, United Kingdom.
71. **Stamm, L. V., and H. L. Bergen.** 2000. A point mutation associated with bacterial macrolide resistance is present in both 23S rRNA genes of an erythromycin-resistant *Treponema pallidum* clinical isolate. Antimicrob. Agents Chemother. **44:**806–807.
72. **Stamm, L. V., H. L. Bergen, and K. A. Shangraw.** 2001. Natural rifampin resistance in *Treponema* spp. correlates with presence of N531 in RpoB rif cluster I. Antimicrob. Agents Chemother. **45:**2973–2974.
73. **Stamm, L. V., and E. A. Parrish.** 1990. In-vitro activity of azithromycin and CP-63,956 against *Treponema pallidum*. J. Antimicrob. Chemother. **25**(Suppl. A)**:**S11–S14.
74. **Stamm, L. V., J. T. Stapleton, and P. J. Bassford, Jr.** 1988. In vitro assay to demonstrate high-level erythromycin resistance in a clinical isolate of *Treponema pallidum*. Antimicrob. Agents Chemother. **32:**164–169.
75. **Stapleton, J. T., L. V. Stamm, and P. J. Bassford, Jr.** 1985. Potential for development of antibiotic resistance in pathogenic treponemes. Rev. Infect. Dis. **7**(Suppl. 2)**:**S314–S317.
76. **Stoner, B. P.** 2007. Current controversies in the management of adult syphilis. Clin. Infect. Dis. **44:**S130–146.
77. **Tichonova, L., K. Borisenko, H. Ward, A. Meheus, A. Gromyko, and A. Renton.** 1997. Epidemics of syphilis in the Russian Federation: trends, origins, and priorities for control. Lancet **350:**210–213.
78. **Tramont, E. C.** 1976. Persistence of *Treponema pallidum* following penicillin G therapy. Report of two cases. JAMA **236:**2206–2207.
79. **Verdon, M. S., H. H. Handsfield, and R. B. Johnson.** 1994. Pilot study of azithromycin for treatment of primary and secondary syphilis. Clin. Infect. Dis. **19:**486–488.
80. **Vester, B., and S. Douthwaite.** 2001. Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrob. Agents Chemother. **45:**1–12.
81. **Wang, G., and D. E. Taylor.** 1998. Site-specific mutations in the 23S rRNA gene of *Helicobacter pylori* confer two types of resistance to macrolide-lincosamide-streptogramin B antibiotics. Antimicrob. Agents Chemother. **42:**1952–1958.
82. **Weigel, L. M., J. D. Radolf, and M. V. Norgard.** 1994. The 47-kDa major lipoprotein immunogen of *Treponema pallidum* is a penicillin-binding protein with carboxypeptidase activity. Proc. Natl. Acad. Sci. U. S. A. **91:**11611–11615.
83. **Wong, T., A. E. Singh, and P. De.** 2008. Primary syphilis: serological treatment response to doxycycline/tetracycline versus benzathine penicillin. Am. J. Med. **121:**903–908.
84. **Woznicova, V., D. Smajs, D. Wechsler, P. Matejkova, and M. Flasarova.** 2007. Detection of *Treponema pallidum subsp. pallidum* from skin lesions, serum, and cerebrospinal fluid in an infant with congenital syphilis after clindamycin treatment of the mother during pregnancy. J. Clin. Microbiol. **45:**659–661.
85. **Wu, J. Y., J. J. Kim, R. Reddy, W. M. Wang, D. Y. Graham, and D. H. Kwon.** 2005. Tetracycline-resistant clinical *Helicobacter pylori* isolates with and without mutations in 16S rRNA-encoding genes. Antimicrob. Agents Chemother. **49:**578–583.
86. **Zapun, A., C. Contreras-Martel, and T. Vernet.** 2008. Penicillin-binding proteins and beta-lactam resistance. FEMS Microbiol. Rev. **32:**361–385.
87. **Zhou, P., Z. Gu, J. Xu, X. Wang, and K. Liao.** 2005. A study evaluating ceftriaxone as a treatment agent for primary and secondary syphilis in pregnancy. Sex. Transm. Dis. **32:**495–498.