AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

CLINICAL THERAPEUTICS

Efficacy of Doxycycline in the Treatment of Syphilis

Ting Dai,[a] Rui Qu,[a] Jinfen Liu,[a] Pingyu Zhou,[b] Qianqiu Wang[c]

Department of Dermatology, Shanghai Xuhui Central Hospital, Shanghai, People's Republic of China[a]; Department of STD Institute, Shanghai Skin Disease Hospital, Shanghai, People's Republic of China[b]; Department of Clinical Management, National Center for STD Control, Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, Jiangsu, People's Republic of China[c]

ABSTRACT Doxycycline is an alternative antibiotic drug for the treatment of syphilis, but data on its efficacy, especially data on its efficacy against late latent syphilis, are limited. A retrospective study was conducted to evaluate the effectiveness of doxycycline for the treatment of patients with different stages of syphilis. Patients who received doxycycline treatment between June 2011 and June 2014 were involved. The serological response to doxycycline was defined as either a negative toluidine red unheated serum test (TRUST) result or a ≥4-fold decrease in titer at 12 months following the treatment. Univariate and multivariate logistic regression analyses were performed to identify factors associated with the serological response. During the study period, a total of 163 syphilis patients were treated with doxycycline, and 118 patients completed doxycycline treatment and the 12-month follow-up. Among the 118 patients, the serological response rate at 12 months was 100.0% (7/7) in patients with primary syphilis, 96.9% (62/64) in patients with secondary syphilis, 91.3% (21/23) in patients with early latent syphilis, and 79.2% (19/24) in patients with late latent syphilis. The total serological response rates were 92.4% (109/118) for preprotocol (PP) patients and 66.9% (109/163) for all intention-to-treat (ITT) patients. In multivariate analysis, patients who serologically responded at 12 months following treatment were positively associated with a higher baseline TRUST titer and an earlier syphilis stage than nonresponders. Our study showed excellent treatment outcomes in patients with different stages of syphilis. Our data, along with those from other reports, support the usage of doxycycline as a good alternative therapeutic option in the treatment of syphilis.

KEYWORDS syphilis, doxycycline, efficacy

Syphilis is a serious infection that can cause acute cutaneous manifestations, chronic compromise of the cardiovascular and nervous systems, and serious effects on reproductive and neonatal health. Syphilis also increases the risk of human immunodeficiency virus (HIV) acquisition and transmission (1). The World Health Organization has estimated that 10 million new infections still occur each year (2). Syphilis therefore remains a worldwide public health problem. Without a vaccine, the efficient diagnosis and the efficient treatment of syphilis are essential for effective syphilis control.

Both U.S. and European guidelines recommend parenterally administered penicillin G for the treatment of all stages of syphilis (3, 4). Although benzathine penicillin is the recommended first-line treatment for syphilis in China, it is not available in many hospitals in China. Doxycycline, tetracycline, and azithromycin were preferred alternative agents in patients allergic to penicillin, especially those who cannot tolerate intramuscular injections. The early success of azithromycin led to considerable enthusiasm (5–7). However, an increased number of azithromycin treatment failures associated with a 23S rRNA mutation for macrolide resistance has been reported from several areas in recent years (8–11). The rate of macrolide resistance is extremely high in China,

Received 27 May 2016 **Returned for modification** 17 July 2016 **Accepted** 5 October 2016

Accepted manuscript posted online 24 October 2016

Citation Dai T, Qu R, Liu J, Zhou P, Wang Q. 2017. Efficacy of doxycycline in the treatment of syphilis. Antimicrob Agents Chemother 61:e01092-16. https://doi.org/10.1128/AAC.01092-16.

Address correspondence to Pingyu Zhou, zpyls@yahoo.com, or Qianqiu Wang, wangqq@ncstdlc.org.

P.Z. and Q.W. contributed equally to this study.

with the 23S rRNA A2058G mutation being present in 91.9% of the patients from a national survey (12). Azithromycin treatment failure was reported in Shanghai, China (13), and our previous study showed that the 23S rRNA A2058G mutation reported in Shanghai ranked as the most frequent mutation in China (14). Tetracycline can cause more gastrointestinal side effects and requires more frequent dosing than doxycycline (15). Therefore, doxycycline is endorsed as an alternative preferred therapy. Regimens of doxycycline of 100 mg orally twice daily for 14 days for early syphilis and 28 days for late syphilis have been used for many years. However, studies of doxycycline for the treatment of syphilis are rare, and their results are partly contradictory (16–21). Early case series reported a very high rate of response to doxycycline treatment, but in most studies the dose was different from the currently recommended dose (16, 17). Recent reports of studies that used the recommended doxycycline dose showed a much lower response rate (18, 21). Most of these reports about doxycycline were from small case series, and none of them included patients with the late stage of syphilis.

The decreased use of macrolides could result in the increased use of tetracyclines or doxycycline. The potential for the development of additional tetracycline or doxycycline resistance due to selective pressure should be a cause for increased concern. To provide more convincing data about doxycycline treatment, we conducted a retrospective study to evaluate the response rates of patients with different stages of syphilis treated with the recommended dose of doxycycline; factors associated with the serological response were also assessed.

RESULTS

Study population. From June 2011 to June 2014, a total of 163 syphilis patients were treated with doxycycline. Of the 163 intention-to-treat (ITT) patients, 129 completed 6 months of follow-up and 118 completed 12 months of follow-up. The 118 patients who completed 12 months of follow-up were included in the study. Of the 45 patients lost to 12 months of follow-up, 19 did not attend any follow-up visits and were excluded from the study (Fig. 1). Of the 19 patients, 3 did not complete doxycycline treatment because of impaired liver function. Among the 118 preprotocol (PP) participants, the median age was 48.0 years (age range, 20 to 69 years), 67 (56.8%) were male, 7 (5.9%) were men who have sex with men (MSM), and 2 (1.7%) were HIV positive. More than half of the patients ($n = 64$, 54.2%) had secondary syphilis, 7 (5.9%) had primary syphilis, 23 (19.5%) had early latent syphilis, and 24 (20.3%) had late latent syphilis (Table 1). Serum toluidine red unheated serum test (TRUST) titers ranged from 1:1 to 1:512 before treatment, with the median titer being 1:32.

Treatment and follow-up. Of the 118 syphilis cases with available treatment outcome data, 94 (79.7%) were treated with doxycycline at 100 mg twice daily for 14 days for early syphilis and 24 (20.3%) were treated with doxycycline at 100 mg twice daily for 28 days for late syphilis. The group with late latent syphilis had a lower median basic serum TRUST titer than the group with early latent syphilis (median TRUST titer, 1:16 versus 1:64). The two treatment groups were generally similar with regard to sociodemographic and behavioral characteristics.

Serological response rate. All the clinical symptoms of primary and secondary syphilis resolved after treatment. The total serological response rates were 92.4% (109/118) for the 118 PP patients. The distribution of the serological response according to the clinical stage of syphilis is shown in Fig. 2. At 12 months, the serological response rate was 100.0% (7/7) in patients with primary syphilis, 96.9% (62/64) in patients with secondary syphilis, 91.3% (21/23) in patients with early latent syphilis, and 79.2% (19/24) in patients with late latent syphilis. Among these patients, the seroreversion rate was 85.7% (6/7) in patients with primary syphilis, 39.1% (25/64) in patients with secondary syphilis, 47.8% (11/23) in patients with early latent syphilis, and 25% (6/24) in patients with late latent syphilis. The median TRUST titer decrease was 16-fold at 12 months. The total serological response rate was 66.9% (109/163) for all ITT patients.

Among the 9 patients with serological failure, 1 patient with late latent syphilis had an 8-fold increase in titers (from 1:4 to 1:32) after therapy, 1 patient with secondary

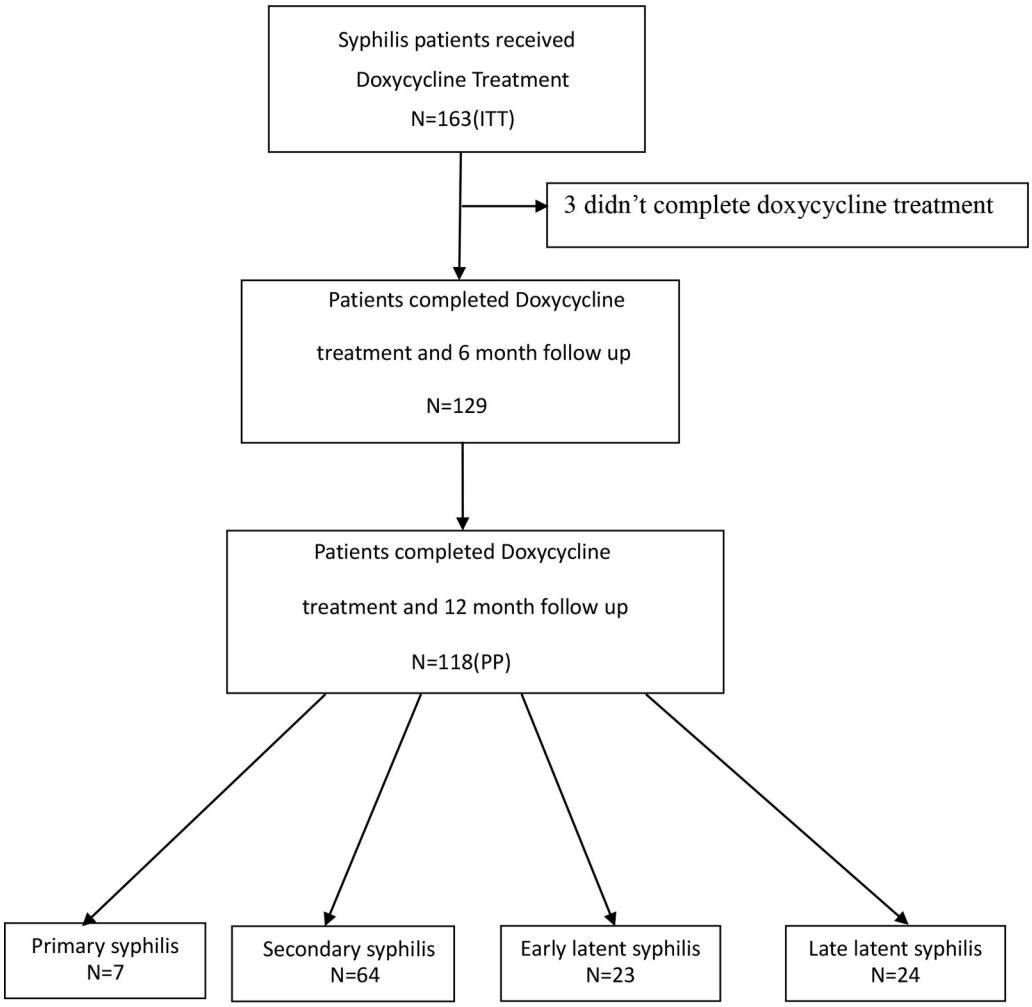

FIG 1 Patient flowchart.

syphilis relapsed at 9 months, and the other 7 patients (1 with secondary syphilis, 2 with early latent syphilis, and 4 with late latent syphilis) did not reach a 4-fold decrease in their titers at 12 months after therapy. Both of the HIV-positive patients achieved a serological response.

Factors associated with serological response. In univariate analysis, patients who serologically responded at 12 months following treatment were positively associated with a higher baseline TRUST titer and an earlier syphilis stage than nonresponders. Multivariate logistic regression analyses also showed that factors positively associated with a serological response were the baseline TRUST titer (>32; adjusted odds ratio [AOR], 18.82; 95% confidence interval [CI], 2.14 to 165.90) and syphilis stage (early AOR, 5.14; 95% CI, 1.04 to 25.37) (Table 2).

DISCUSSION

The evidence upon which recommendations for syphilis therapy are based remains inadequate. The major advantages of benzathine penicillin treatment are its safety, effectiveness, and favorable adherence to the weekly dosing schedule. The factor most limiting the use of benzathine penicillin in China is its availability, though penicillin allergy remains an issue throughout the world. Doxycycline is a tetracycline derivative with better oral bioavailability than tetracycline, convenient twice-a-day dosing, and fewer gastrointestinal side effects (15, 22). As an alternative treatment, doxycycline presents several advantages.

Our study showed excellent treatment outcomes in early syphilis patients (re-

TABLE 1 Demographic, clinical, and laboratory data for the syphilis patients

Characteristic	% of patients (no. of patients with the characteristic/total no. tested)
Sex	
Male	56.8 (67/118)
Female	43.2 (51/118)
Serum TRUST titer	
≤8	13.6 (16/118)
16–32	39.8 (47/118)
≥64	46.6 (55/118)
Sexual orientation	
MSM[a]	5.9 (7/118)
Heterosexual	94.1 (111/118)
Stage of syphilis	
Primary	5.9 (7/118)
Secondary	54.2 (64/118)
Early latent	19.5 (23/118)
Late latent	20.3 (24/118)
Age (yr)	
≤35	22.0 (26/118)
36–50	36.4 (43/118)
>50	41.5 (49/118)

[a]MSM, men who have sex with men.

sponse rates, 100.0% in patients with primary syphilis, 96.9% in patients with secondary syphilis, and 91.3% in patients with early latent syphilis). The findings are consistent with those of early retrospective studies by Onoda (16), Harshan and Jayakumar (17), and Ghanem et al. (18). Both Harshan and Jayakumar (17) and Ghanem et al. (18) reported a 100% response rate in patients with early syphilis. Similarly, 14 of 15 (93.3%) early syphilis patients had serological evidence of a response to treatment in the study of Onoda (16). It should be pointed out that in the study of Onoda (16), patients were treated with repeated doxycycline therapy at a dosage double that used in the current study, while in the study of Harshan and Jayakumar (17), patients were treated with one-half the dosage of doxycycline used in the current study. However, the serological response rate seemed to be much lower in recently published studies in which patients were treated with the same dosage of doxycycline used in the current study (19–21). The response rate was

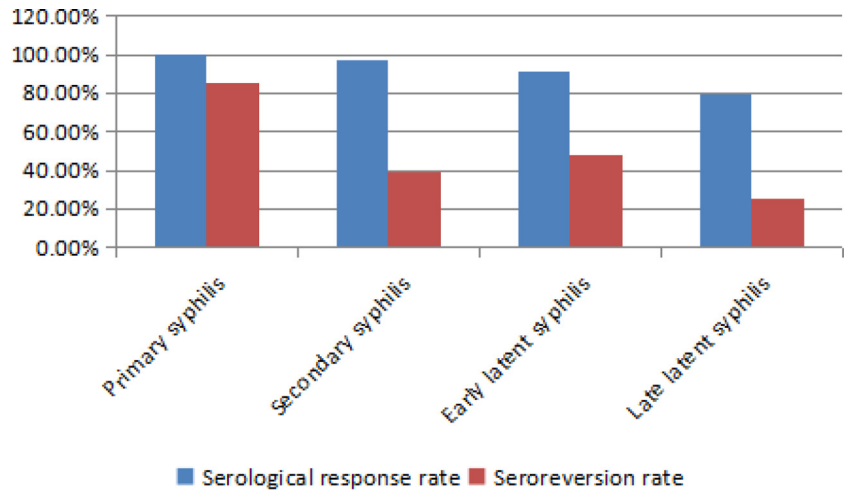

FIG 2 Serological response rates and seroreversion rates at 12 months following treatment.

TABLE 2 Univariate analysis of factors associated with serological response at 12 months following treatment[a]

Variable	Univariate OR (95% CI)	Multivariate AOR (95% CI)
Sex		
Male	1.71 (0.44–6.73)	
Female	1	
Age (yr)		
≤35	0.50 (0.09–2.67)	
35–50	0.87 (0.17–4.55)	
>50	1	
Serum TRUST titer		
≤16	1	1
>32	28.33 (3.38–237.86)**	18.82 (2.14–165.90)**
Stage of syphilis		
Early	10.11 (2.31–44.21)**	5.14 (1.04–25.37)**
Late	1	1
MSM		
Yes	1	
No	2.15 (0.23–20.07)	

[a]CI, confidence interval; OR, odds ratio; AOR, adjusted odds ratio; **, $P < 0.01$.

63.4% and 82.9% in early syphilis in the studies of Tsai et al. (21) and Li and Zheng (20), respectively. This broad range may be related to the different patient cohorts in each study, in addition to the different methods adopted by each study. Most studies suggest a higher probability of serological failure for patients with more advanced syphilis, HIV infection, and a history of syphilis (23, 24). The differences in the results of the different studies could thus be explained accordingly. On the one hand, almost all the patients in our study were HIV negative and had no history of syphilis, while in the previous studies, HIV-infected patients accounted for 11% to 100% of the patients enrolled. Because those with HIV infection have an increased risk of serological failure, their HIV infection status may have increased the failure rate. On the other hand, the number of male patients in our study was similar to the number of female patients, and most of these patients were married and received syphilis treatment together with their spouse; thus, the rates of recurrent exposure to syphilis in the sexual network and reinfection might have been lower. Furthermore, our study mainly analyzed patients who completed 12 months of follow-up, and patients lost to follow-up were excluded. If the data for all 163 intention-to-treat patients are considered, the success rate would have been much lower (66.9%, 109/163).

There are few data about doxycycline treatment in late latent syphilis patients. Our study showed that the response rate was 79.2%. Of note, most of the treatment failures occurred in patients with TRUST titers of ≤8. For patients with late latent syphilis, most patients had received antibiotics for other conditions, and the TRUST titer could have decreased before syphilis-specific treatment. In multivariate regression analysis, the baseline TRUST titer and syphilis stage were significantly associated with the serological response. Our findings generally support those of other investigations regarding the relationship between the baseline rapid plasma reagin test titers and the serological response (25). Only two of the nine patients with treatment failure showed a relapse.

This study has several limitations. First, benzathine penicillin was not available in our hospital during the study period, and the absence of a control group treated with benzathine penicillin G is a major concern. Second, the clinical data were collected from a single center and the study was retrospective; there were no standardized criteria for the choice of an alternative therapy. Third, the patients included in the study were

those who had documented follow-up serological tests. The results were far from perfect because of the loss of patients to follow-up. Therefore, both selection and information biases should be acknowledged. Fourth, the length of infection in some of the late latent syphilis patients was unknown and patients might have still been in the early infectious stage, as those patients had high TRUST titers. The strengths of our study are that it had a relatively large sample size of syphilis patients treated with doxycycline and it included patients with late latent syphilis. Overall, our data, along with those from other studies, support the usage of doxycycline as a good alternative therapeutic option in the treatment of syphilis.

MATERIALS AND METHODS

The first-line medicine benzathine penicillin was not available at Shanghai Xuhui Central Hospital during the study period. Doxycycline was therefore given to syphilis patients as an alternative therapy. The subjects included in the study were consecutive adults ≥18 years of age who were diagnosed with syphilis and agreed to treatment with doxycycline for syphilis at the sexually transmitted disease (STD) clinic of the Shanghai Xuhui Central Hospital (Shanghai Clinical Center, Chinese Academy of Sciences) between June 2011 and June 2014. Information on the patients' demographic characteristics, symptoms, sexual orientation, stage of syphilis, HIV-1 infection status, treatment, and serological test results were collected using a standardized clinical form. The diagnosis of primary, secondary, early latent, and late latent syphilis was made by trained clinicians. Primary syphilis is characterized by an ulcer or chancre and laboratory confirmation of the presence of *Treponema pallidum* by positivity by both the *Treponema pallidum* particle assay (TPPA) and the nontreponemal toluidine red unheated serum test (TRUST). Secondary syphilis is generally characterized by a skin rash and mucocutaneous lesions, usually with lymphadenopathy, and was confirmed by laboratory tests. Latent syphilis was defined as an asymptomatic case detected by reactive TRUST and TPPA results. Latent syphilis acquired within the preceding year was classified as early latent syphilis; other cases of unknown duration or of more than 12 months in duration are referred to as late latent syphilis.

Laboratory tests. Laboratory analyses, including the serum TRUST and TPPA, were conducted in accordance with the instructions of the manufacturers. The TRUST reagents were manufactured by Shanghai Rongsheng Biotech, and the TPPA reagents were manufactured by Fujirebio Inc. All serum samples used in the TRUST were diluted to avoid prozone effects and false-negative results. The titers of the serum samples that were reactive by TRUST were quantified using 2-fold serial dilutions until the endpoint was determined. The HIV serological status of the eligible participants was determined by a dual enzyme-linked immunosorbent assay.

Treatment and follow-up. Doxycycline therapy consisted of 100 mg orally twice daily for 14 days for early syphilis and 28 days for late latent syphilis. As a routine in the STD clinic of the Shanghai Xuhui Central Hospital, after treatment, all the patients were asked to have their clinical symptoms periodically reviewed and to have their serum TRUST titers determined every 3 months in the first year and every 6 months in the following years. Serological response criteria were defined as either a negative TRUST result or a ≥4-fold decrease in titer at 12 months following treatment. Seroreversion meant that a positive TRUST result became negative after treatment. To be included in the study, at least two visits (one visit for determination of an initial titer at the time of treatment and at least one follow-up visit for determination of the titer 12 months after the end of treatment) were necessary for the observation of serological titer changes. On the other hand, the serological response was calculated by considering all the intention-to-treat patients.

Statistical methods. Data were recorded using Microsoft Excel software and validated through double entry. Univariate and multivariate logistic regression analyses were performed using the Statistical Package for the Social Sciences (version 17.0; SPSS Inc., Chicago, IL, USA) to identify factors associated with the serological response. Odds ratios (ORs) and their corresponding 95% CIs were produced to determine factors associated with the serological response. Multiple regression analyses were performed to adjust the ORs for potential confounders. Only variables that were significant in the univariate analyses were included in the multivariable logistic regression models for the selection of independent risk factors. The Ethical Committee of the Shanghai Xuhui Central Hospital (Shanghai Clinical Center, Chinese Academy of Science) waived the need for institutional review board approval because of the retrospective nature of this study.

ACKNOWLEDGMENTS

We thank the specialists from the Eye & ENT Hospital of Fudan University who initially diagnosed the syphilis in the study patients and all our colleagues who helped to manage these patients.

This study was supported by the Jiangsu Provincial Special Fund for Clinical Sciences and Technology (BL2012003), the Shanghai Health and Family Planning Commission (2013SY068), and Shanghai Xuhui Central Hospital research project 2015XHYY-07.

REFERENCES

1. Golden MR, Marra CM, Holmes KK. 2003. Update on syphilis: resurgence of an old problem. JAMA 290:1510–1514. https://doi.org/10.1001/jama.290.11.1510.
2. World Health Organization. 2011. Global HIV/AIDS response: epidemic update and health sector progress towards universal access: progress report. World Health Organization, Geneva, Switzerland.
3. Janier M, Hegyi V, Dupin N, Unemo M, Tiplica GS, Potočnik M, French P, Patel R. 2014. 2014 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol 28:1581–1593. https://doi.org/10.1111/jdv.12734.
4. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. 2015. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recommend Rep 64(RR-03):1–137.
5. Hook EW, III, Martin DH, Stephens J, Smith BS, Smith K. 2002. A randomized, comparative pilot study of azithromycin versus benzathine penicillin G for treatment of early syphilis. Sex Transm Dis 29:486–490. https://doi.org/10.1097/00007435-200208000-00010.
6. Riedner G, Rusizoka M, Todd J, Maboko L, Hoelscher M, Mmbando D, Samky E, Lyamuya E, Mabey D, Grosskurth H, Hayes R. 2005. Single-dose azithromycin versus penicillin G benzathine for the treatment of early syphilis. N Engl J Med 12:1236–1244.
7. Hook EW, III, Behets F, Van Damme K, Ravelomanana N, Leone P, Sena AC, Martin D, Langley C, McNeil L, Wolff M. 2010. A phase III equivalence trial of azithromycin versus benzathine penicillin for treatment of early syphilis. J Infect Dis 11:1729–1735. https://doi.org/10.1086/652239.
8. Mitchell SJ, Engelman J, Kent CK, Lukehart SA, Godornes C, Klausner JD. 2006. Azithromycin-resistant syphilis infection: San Francisco, California, 2000-2004. Clin Infect Dis 42:337–345. https://doi.org/10.1086/498899.
9. Martin IE, Tsang RS, Sutherland K, Tilley P, Read R, Anderson B, Roy C, Singh AE. 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. https://doi.org/10.1128/JCM.02392-08.
10. Van Damme K, Behets F, Ravelomanana N, Godornes C, Khan M, Randrianasolo B, Rabenja NL, Lukehart S, Cohen M, Hook E. 2009. Evaluation of azithromycin resistance in Treponema pallidum specimens from Madagascar. Sex Transm Dis 36:775–776. https://doi.org/10.1097/OLQ.0b013e3181bd11dd.
11. Tipple C, McClure MO, Taylor GP. 2011. High prevalence of macrolide resistant Treponema pallidum strains in a London centre. Sex Transm Infect 87:486–488. https://doi.org/10.1136/sextrans-2011-050082.
12. Chen XS, Yin YP, Wei WH, Wang HC, Peng RR, Zheng HP, Zhang JP, Zhu BY, Liu QZ, Huang SJ. 2013. High prevalence of azithromycin resistance to Treponema pallidum in geographically different areas in China. Clin Microbiol Infect 19:975–979. https://doi.org/10.1111/1469-0691.12098.
13. Zhou P, Li K, Lu H, Qian Y, Gu X, Gong W, Tucker JD, Cohen MS. 2010. Azithromycin treatment failure among primary and secondary syphilis patients in Shanghai. Sex Transm Dis 37:726–729. https://doi.org/10.1097/OLQ.0b013e3181e2c753.
14. Lu H, Li K, Gong W, Yan L, Gu X, Chai Z, Guan Z, Zhou P. 2015. High frequency of 23S rRNA A2058G mutation of Treponema pallidum in Shanghai is associated with a current strategy for the treatment of syphilis. Emerg Microbes Infect 4:e10. https://doi.org/10.1038/emi.2015.10.
15. Arcilla JD, Fiore JL, Jr, Resnick O, Nadelmann JW, Huth JL, Troetel WM. 1974. Comparative bioavailability of doxycycline. Curr Ther Res Clin Exp 16:1126–1136.
16. Onoda Y. 1979. Therapeutic effect of oral doxycycline on syphilis. Br J Vener Dis 55:110–115.
17. Harshan V, Jayakumar W. 1982. Doxycycline in early syphilis: a long term follow up. Indian J Dermatol 27:119–124.
18. Ghanem KG, Erbelding EJ, Cheng WW, Rompalo AM. 2006. Doxycycline compared with benzathine penicillin for the treatment of early syphilis. Clin Infect Dis 42:e45119–e49. https://doi.org/10.1086/500406.
19. Psomas KC, Brun M, Causse A, Atoui N, Reynes J, Le Moing V. 2012. Efficacy of ceftriaxone and doxycycline in the treatment of early syphilis. Med Mal Infect 42:15–19. https://doi.org/10.1016/j.medmal.2011.10.003.
20. Li J, Zheng HY. 2014. Early syphilis: serological treatment response to doxycycline/tetracycline versus benzathine penicillin. J Infect Dev Ctries 8:228–232. https://doi.org/10.3855/jidc.3013.
21. Tsai JC, Lin YH, Lu PL, Shen NJ, Yang CJ, Shen NJ, Yang CJ, Lee NY, Tang HJ, Liu YM, Huang WC, Lee CH, Ko WC, Chen YH, Lin HH, Chen TC, Hung CC. 2014. Comparison of serological response to doxycycline versus benzathine penicillin G in the treatment of early syphilis in HIV-infected patients: a multi-center observational study. PLoS One 9:e109813. https://doi.org/10.1371/journal.pone.0109813.
22. Petzoldt D. 1972. Doxycycline in the single-dose treatment of gonorrhoea. Effect on Treponema pallidum. Br J Vener Dis 48:514–516.
23. Gonzalez-Lopez JJ, Guerrero ML, Lujan R, Tostado SF, de Gorgolas M, Requena L. 2009. Factors determining serologic response to treatment in patients with syphilis. Clin Infect Dis 49:1505–1511. https://doi.org/10.1086/644618.
24. Ghanem KG, Erbelding EJ, Wiener ZS, Rompalo AM. 2007. Serological response to syphilis treatment in HIV-positive and HIV-negative patients attending sexually transmitted diseases clinics. Sex Transm Infect 83:97–101. https://doi.org/10.1136/sti.2006.021402.
25. Marra CM, Maxwell CL, Smith SL, Lukehart SA, Rompalo AM, Eaton M, Stoner BP, Augenbraun M, Barker DE, Corbett JJ, Zajackowski M, Raines C, Nerad J, Kee R, Barnett SH. 2004. Cerebrospinal fluid abnormalities in patients with syphilis: association with clinical and laboratory features. J Infect Dis 189:369–376. https://doi.org/10.1086/381227.