High prevalence of azithromycin resistance to *Treponema pallidum* in geographically different areas in China

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Abstract

Treatment with effective antibiotics is one important strategy for syphilis control in China. This study aimed to evaluate the prevalence of azithromycin resistance to *T. pallidum* in China. A cross-sectional study was conducted among 391 patients with early syphilis recruited from STD clinics in eight cities during October 2008 and October 2011. The swabs were obtained from the moist lesions of the participating patients. A touchdown/nested PCR of the 23S ribosomal RNA (rRNA) gene was performed on DNA samples extracted from these specimens. The presence or absence of the A2058G point mutation, conferring resistance to azithromycin, was determined by restriction enzyme digestion analysis of the PCR amplicon by MboII. Two hundred and eleven patients with primary or secondary syphilis were found to have *T. pallidum* DNA in their moist lesions by PCR assays. The A2058G mutation was present in 91.9% (194/211, 95% CI, 87.2–95.1%) of these patients, with no significant differences noted between patients from the eastern part (93.8%), southern part (88.6%) and northern part (95.2%) of China ($\chi^2 = 2.303$, p 0.316). Compared with patients who had not taken macrolides in previous years before study entry, the patients who had taken the antibiotics had a significantly higher prevalence of azithromycin resistance (97.0% vs. 62.5%), with an odds ratio of 19.65 (95% CI, 5.77–66.93). It can be concluded that prevalence of azithromycin resistance is substantial in China and consequently that the macrolides should not be used as a treatment option for early or incubating syphilis in China.

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Introduction

Syphilis, a chronic infectious disease caused by the spirochaete *Treponema pallidum* and usually transmitted through sexual contact or from mother to baby, remains a significant public health problem globally. The World Health Organization (WHO) estimated that there were 11 million new cases of syphilis worldwide in 2005, and the majority of them occurred in developing countries [1]. Since the 1980s, this disease has made a strong resurgence in China, exceeding that seen in other countries. Recent data from national surveillance systems have shown that the rate of syphilis has jumped from 6.1 cases per 100 000 people in 2001 to 32.0 per 100 000 in 2011 [2]. In several major cities such as Shanghai in China, it is now the leading notifiable infectious disease.

Treatment with effective antibiotics is one important strategy for syphilis control. Although single intramuscular injection of 2.4 million units (MU) of penicillin G benzathine is the recommended therapy for early syphilis, azithromycin has been used as an alternative or the third-line therapy in patients who are allergic to penicillin in some countries [3,4] because it has the advantage of a single oral dose administration rather than intramuscular injection, which allows patients to deliver the therapy to their sexual partners, and outreach workers to

deliver the therapy in high-risk population settings to improve treatment coverage. A recently published systematic review on the basis of three randomized controlled trials indicates no statistically significant difference between azithromycin and penicillin G benzathine in relative effectiveness for treatment of early syphilis [5]. Meanwhile, monitoring for the resistances of *T. pallidum* strains to macrolide antibiotics and azithromycin treatment failures in patients with syphilis has already attracted widespread attention in many countries, including China. A high prevalence of azithromycin-resistant strains and treatment failure has been reported among 39 patients with primary and secondary syphilis in Shanghai [6], while no resistance was found among 211 clinical specimens from 136 patients with syphilis in Taiwan [7].

In this study, we aimed to investigate the prevalence of azithromycin-resistant *T. pallidum* among patients with early syphilis at sexually transmitted disease (STD) clinics in geographically different areas in China.

Methods

Study areas and participants

The study was conducted in eight cities in the east (Nanjing), south (Nanning, Guangzhou, Jiangmen, Fuzhou and Chengdu) and north (Harbin and Tianjin) parts of China during October 2008 and October 2011. Geographical locations of the study sites are shown in Figure 1. Criteria for participating in the study included: being patients attending the STD clinics in the study cities; having moist lesions (chancres or condyloma lata) consistent with diagnosis of primary or secondary syphilis according to the national diagnosis guidelines [6]; having not received any antibiotics for current syphilis infection before enrollment; and having ability to give an informed consent. After signing an informed consent form, the eligible patients were interviewed to obtain socio-demographic and clinical data and then underwent a blood and moist lesion specimen collection for serological and molecular biology tests. The study protocols were reviewed and approved by the Medical Ethics Committee of the Chinese Academy of Medical Sciences Institute of Dermatology in Nanjing, China.

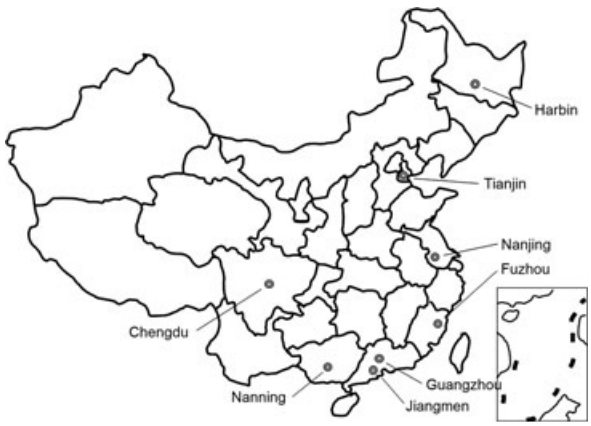

FIG. 1. Geographical locations of the study sites in China.

Clinical specimens

All participants were requested to provide serum specimens for syphilis serological testing and swab specimens for molecular evaluation of *T. pallidum*.

Venous blood samples were obtained from 391 patients with early syphilis. Swabs were also taken from the moist lesions of these patients. Swab specimens were stored in sterile containers at −70°C until they were transported to the National STD Reference Laboratory at the National Centre for STD Control in Nanjing for molecular evaluation there.

Dark-field microscopy and serological tests

Exudates from ulcerative lesions were collected onto a clean microscopic slide by placing the exudate directly on the slide and were immediately examined under the dark-field microscopy by an experienced technician at the local STD clinics. Serum samples from recruited patients were simultaneously tested using a non-treponemal test (Toluidine Red Unheated Serum Test (TRUST); Shanghai Rongsheng Biotech, Shanghai, China) and a treponemal test (*Treponema pallidum* particle agglutination (TPPA); Fujirebio, Tokyo, Japan). Those specimens with reactive TRUST further underwent a quantitative TRUST testing. All the serological tests for syphilis were conducted in the local STD clinics.

Detection of azithromycin resistance

Extraction of DNA from clinical specimens was accomplished using the Qiagen QIAamp® mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The PCR assay of the polA gene was performed based on a previously reported method [8]. The presence or absence of the A2058G point mutation, conferring resistance to azithromycin, was determined by touchdown/nested polymerase chain reaction amplification (PCR) of the 23S ribosomal RNA (rRNA) gene, followed by restriction-digestion analysis of the amplicon by *MboII*, as has been described previously [9]. The DNA extraction and PCR detection was conducted in the National STD Reference Laboratory.

Statistical analysis

All data from the interview and the laboratory results were concurrently double-entered into a computer database using EpiData Software (version 3.0) by independent research