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ORIGINAL RESEARCH

Molecular Characteristics of Macrolide Resistance in *Treponema pallidum* from Patients with Latent Syphilis in Xinjiang, China

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Background: Macrolide resistance in *Treponema pallidum* (*T. pallidum*) has been increasing in recent years worldwide. However, few data are available on macrolide resistance in *T. pallidum* from Xinjiang province, located in the western part of China, which is an area with a high incidence of syphilis. In this study, we investigated the molecular characteristics of macrolide resistance in *T. pallidum* from patients with latent syphilis in Xinjiang, China.

Methods: In total, 204 whole blood samples were collected from patients with latent syphilis during 2016 to 2017, in the First Hospital of Xinjiang Medical University. Genomic DNA of blood samples was extracted using a QIAamp DNA Mini Kit and *T. pallidum* was detected by PCR with the specific *polA* gene of *T. pallidum*. The 23S rRNA gene of *T. pallidum* was amplified among the *T. pallidum*-positive samples by nested PCR, and macrolide resistance-associated mutation sites A2058G and A2059G in the 23S rRNA gene were identified using restriction enzymes *Mbo*II and *Bsa*I.

Results: The specific *polA* gene of *T. pallidum* (*T. pallidum* positive) was detected in 27 blood samples (13.2%) from 204 patients with latent syphilis. The 23S rRNA gene was then amplified in all 27 *T. pallidum*-positive samples, among which 24/27 samples (88.9%) harbored the A2058G mutation in the 23S rRNA gene and 3/27 (11.1%) had the A2059G mutation.

Conclusion: Our results indicated that *T. pallidum* macrolide resistance should not be ignored in Xinjiang, China, and that A2058G was the predominant macrolide resistance mechanism. Blood may be a suitable specimen for the detection of resistant mutations of *T. pallidum* in patients with latent syphilis who do not show any clinical symptoms.

Keywords: macrolide resistance, *Treponema pallidum*, latent syphilis, 23S rRNA

Introduction

Syphilis is a chronic, multistage, sexually transmitted infection(STI) caused by *Treponema pallidum*. Latent syphilis is asymptomatic, but with positive serological reactions to *T. pallidum*.[1] In China, incidence rates of syphilis increased from 7.12/100,000 in 2004 to 31.97/100,000 in 2016. Of note, latent syphilis accounts for about 80% of cases.[2,3] The high incidence of patients with latent syphilis may be attributed to the extensive syphilis screening programs in the medical institutions. Xinjiang was the region with the highest incidence of syphilis (89.05%, 1/100,000) in China, where latent syphilis accounts for 80% as well, according to a report in 2016.[2,3]

Macrolide resistance in *T. pallidum* and clinical treatment failure have emerged rapidly worldwide where macrolides have been used as an alternative treatment for syphilis, as well as for other infections.[4,5] Previous studies indicated that A2058G mutation and A2059G mutation in the 23S rRNA gene of *T. pallidum* are common mechanisms causing macrolide resistance in *T. pallidum*.[6,7] In China, high rates of A2058G mutation have been reported in Shanghai (95.4%),[8] Hunan (97.5%),[9] Shangdong (92.1%),[10] and Xiamen (100%),[11] whereas the A2059G mutation in the 23S rRNA gene of *T. pallidum* has only been reported in Shangdong, China, by Li et al.[10] Taking swab samples from

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chancres was the most common method used to isolate *T. pallidum* for the detection of A2058G and A2059G point mutations in the 23S rRNA gene of *T. pallidum* in previous studies.[12–14] However, it is impossible to isolate *T. pallidum* from chancres in patients with latent syphilis, who have an absence of chancres and the other syphilis skin lesions. Several studies have reported using blood samples from syphilis patients to detect *T. pallidum* and macrolide resistance mutations in *T. pallidum*, although the detection rate of *T. pallidum* DNA is relatively low.[9,15] Latent syphilis is the most common clinical type in Xinjiang,[3] but few data are available on the prevalence of *T. pallidum* resistance to macrolides in these patients. The aim of this study was to detect A2058G and A2059G mutations in the 23S rRNA gene of *T. pallidum* using blood samples from patients with latent syphilis to investigate the molecular characteristics of macrolide resistance in *T. pallidum* in Xinjiang.

Materials and Methods

Collection of Clinical Specimens

A total of 204 whole blood samples were collected from 204 patients diagnosed with latent syphilis from March 2016 to October 2017 at the First Hospital of Xinjiang Medical University in Xinjiang, China. The diagnosis of latent syphilis was based on the treatment guidelines for sexually transmitted diseases (2015);[16] namely, no obvious clinical signs and symptoms related to syphilis and both rapid plasma reagin test (RPR) (titer >1:1) and *T. pallidum* hemagglutination (TPHA) positive (+).

Detection of *T. pallidum*

Genomic DNA was isolated from the whole blood samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). For the detection of *T. pallidum*, the specific *polA* gene of *T. pallidum* was amplified by PCR, as described previously,[17] and positive samples with the specific *polA* gene of *T. pallidum* were determined with a 377-bp fragment presenting on agarose gel electrophoresis. The primer sequences used are listed in Table S1. PCR was performed under the following cycling conditions: 94°C (3 min); 94°C (30 s); 66°C (30 s) and 72°C (30 s) for 38 cycles; and 72°C (7 min).

Identification of A2058G and A2059G Mutations in 23S rRNA Genes

Amplification of 23S rRNA genes in *the T. pallidum*-positive sample was conducted by nested PCR, as described previously.[18] The primer sequences used are listed in Table S1. PCR amplification of 23S rRNA was performed under the following cycling conditions: 94°C (5 min); 94°C (1 min); 56°C (1 min) and 72°C (1 min) for 40 cycles; and 72°C (7 min). The second step of nested PCR was performed using touchdown PCR. Cycling conditions were 94°C for 5 min; three cycles of 94°C for 1 min; 55–65°C for 1 min; 72°C for 1 min; and a final extension at 72°C for 1 min. The 491-bp band or 629-bp band presenting on agarose gel indicated positivity for 23S rRNA genes. Furthermore, A2058G and A2059G mutations in 23S rRNA genes were identified by restriction enzyme digestion using *Mbo*II and *Bsa*I enzymes, as described previously.[19] The enzyme digestion reaction was carried out in incubators at 37°C for 16 hours. The digestion products were separated on 1.5% agarose gels to identify the A2058G mutation site with a double band (191 bp +300 bp) and A2059G with a double band (197 bp + 432 bp).[19] The mutation sites were recognized by sequencing. The enzymes used and the mutation sites recognized by restriction digestion analysis in the 23S rRNA gene are listed in Table 1.

Table 1 Enzymes Used and Mutation Sites Recognized by Restriction Enzyme Digestion Analysis in the 23S rRNA Gene

Mutation Point	Type of Digestion	Sequences	Recognized Segments	Product Size (bp)
A2058G	*Mbo*II	AGACGGGAAGACCCC	GAAGA	191 and 300
A2059G	*Bsa*I	TAGACGGAGAGACCCC	GAGACC	197 and 432

Table 2 Results of Serology Tests and Molecular Detection for *Treponema pallidum*

Gender (n)	RPR (%)			TPHA (%)		PCR(+) (n)
	()	≤1:8	≥1:16	()	(+)	
Male (109)	0	16	84	0	100	15
Female (95)	0	28	72	0	100	12
Total						27

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval for the study was obtained from the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. All patients consented to being involved in this study.

Results

The clinical and epidemiological characteristics of the 204 patients with latent syphilis in this study are summarized in Table S2. Table 2 shows the results of serology tests for *T. pallidum*. Figure 1A shows the amplification (377-bp band) of the *T. pallidum*-specific *polA* gene for partial clinical samples. The *T. pallidum*-specific *polA* gene was amplified successfully in 27 samples among the 204 whole blood samples recovered from patients with latent syphilis, which indicates that 27 blood samples contained *T. pallidum*, and thus the detection rate of *T. pallidum* was 13.2% (27/204). Furthermore, as shown in Figure 1B and C, the 23S rRNA gene was identified in these 27 *T. pallidum*-positive samples.

With regard to mutant site detection in the 23S rRNA mutation gene among the 27 *T. pallidum*-positive samples, 24 samples contained the A2058G point mutation (24/27, 88.9%) as shown in Figure 1D, and three samples (3/27, 11.1%) contained the A2059G point mutation, as shown in Figure 1E.

Discussion

In this study, we collected 204 blood sample from patients diagnosed with latent syphilis in Xinjiang. *Treponema pallidum* was detected in 27 samples from 204 blood samples of patients with latent syphilis (Table 2, Figure 1A). The positive detection rate of *T. pallidum* was therefore 13.2% (27/204). Our results confirmed that the positive rate for amplification of *T. pallidum* from whole blood samples was lower than that from chancre swab samples, owing to the low *T. pallidum* burden in blood samples.[20] However, Xiao et al[9] assessed 2253 whole blood samples of patients with secondary and latent syphilis by PCR, using four specific gene markers (*polA, tpp47, bmp*, and *tp0319*); *T. pallidum* was detected in 455 blood samples (20.2%, 455/2253), which was higher than the rate in our study. This may be due to Xiao et al using four specific target genes to detect *T. pallidum*, which increased the detection rate of *T. pallidum*. Thus, the detection rate of *T. pallidum* could be improved by using an optimal PCR approach and increasing the number of specific target genes.[7,20]

Nevertheless, among 27 *T. pallidum*-positive samples, the A2058G mutation was identified in 24 samples (88.9%) (Figure 1D) and the A2059G mutation was found in three samples (11.1%) (Figure 1E). These findings indicate that macrolide resistance in *T. pallidum* exists in patients with latent syphilis in Xinjiang, and the A2058G mutation is the predominant mechanism of macrolide resistance in *T. pallidum*, which is consistent with the results reported in previous studies in Shanghai (95.4%),[8] Hunan (97.5%),[9] Shangdong (92.1%),[10] and Xiamen (100%).[11] In China, the A2059G mutation in the 23S rRNA gene of *T. pallidum* has been reported only in Shangdong, with a 7.6% incidence;[10] however, it was not detected in Shanghai[8] or Hunan province.[9] In our study, the mutation rate of A2059G was 11.1% (3/27) in the Xinjiang area, which was higher than that reported in Shangdong (7.6%); the reason for this result may be that the prevalence of the A2059G mutation varies between geographic areas and populations. Fernández-Naval et al[15] investigated macrolide resistance in *T. pallidum* among 130 *T. pallidum*-positive samples, including 88 chancre swab samples and 42 blood samples, from Barcelona; the A2058G mutation was identified in 99% of samples and the A2059G mutation was found only in one sample. Thus, those findings confirmed that the A2058G point mutation is the most

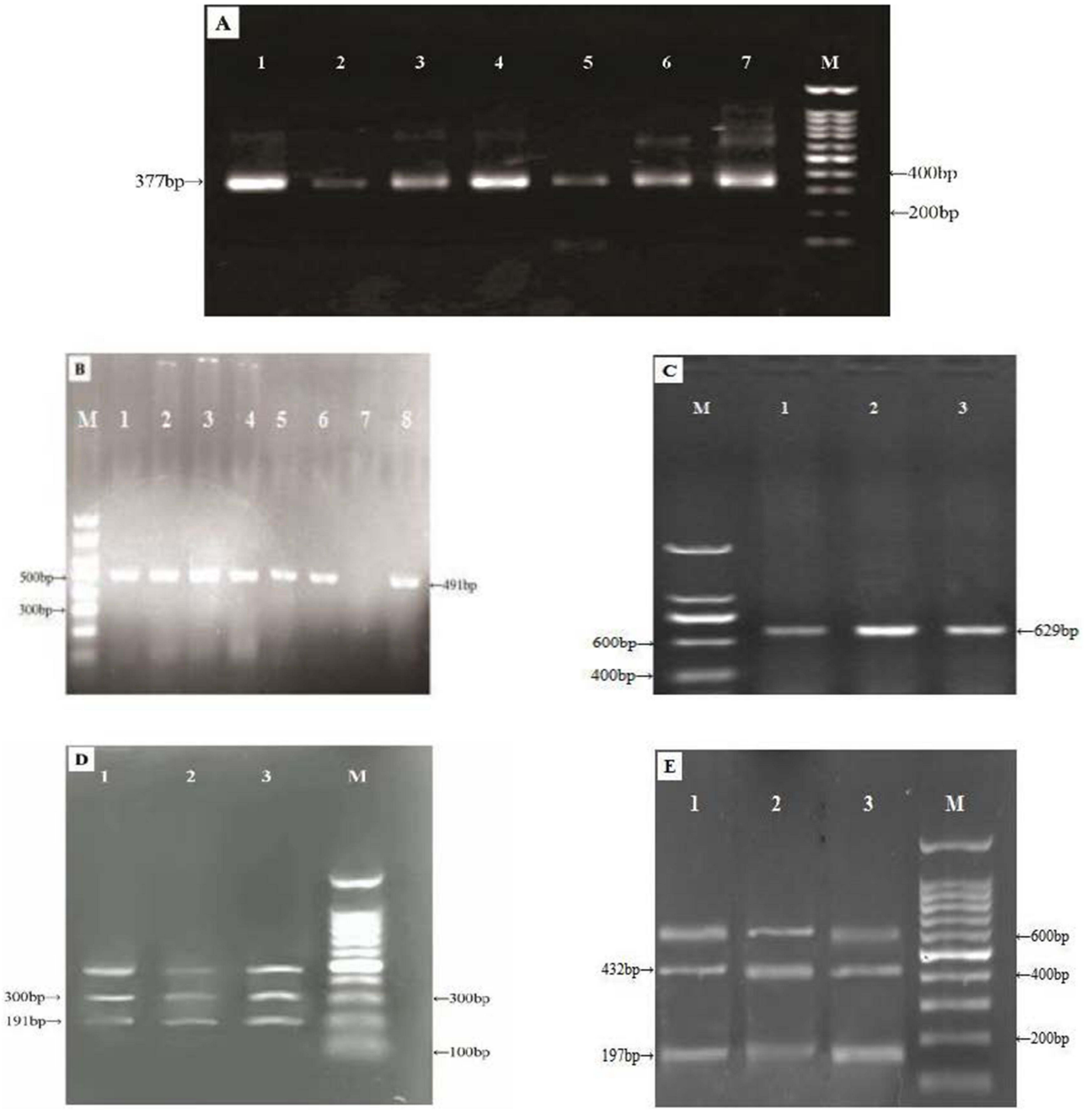

Figure 1 Images of agarose gel electrophoresis for each PCR amplification result. (**A**) The amplification result for *T. pallidum* -specific *polA* gene (377 bp) on agarose gel electrophoresis. M, 100-bp DNA ladder; lane 1, *T. pallidum* Nichols strain; lanes 2–7, *T. pallidum* samples from patients with latent syphilis from Xinjiang. (**B**) Agarose gel electrophoresis of nested PCR of 23S rRNA gene. M, 200-bp DNA ladder; lanes 1–6, uncut clinical isolate DNA (491 bp, A2058G) from patients with latent syphilis from Xinjiang; lane 7, negative control; lane 8, uncut strain SS14 DNA (491 bp, A2058G). (**C**) M, 200-bp DNA ladder; lanes 1–3, uncut clinical isolate DNA (629 bp, A2059G) from patients with latent syphilis from Xinjiang. (**D**) Agarose gel electrophoresis of restriction enzyme digestion products after nested PCR of the 23S rRNA gene. Lane 1, *Mbo*II digestion (191+300 bp) of strain SS14 DNA (A2058G); lanes 2 and 3, *Mbo*II digestion (191+300 bp) of clinical isolate DNA (A2058G) from patients with latent syphilis from Xinjiang; M, 100-bp DNA ladder. (**E**) Agarose gel electrophoresis of restriction enzyme digestion products after nested PCR of 23S rRNA gene. Lanes 1–3, *Bsa*I digestion (197+432 bp) of clinical isolate DNA (A2059G) from patients with latent syphilis from Xinjiang; M, 100-bp DNA ladder.

frequent resistance mutation, whereas the A2059G mutation is uncommon as a mechanism of macrolide resistance in *T. pallidum*. However, the genetic origin and mechanisms leading to *T. pallidum* macrolide resistance are poorly understood, and further research is needed.

Several studies have reported that the prevalence of macrolide resistance in *T. pallidum* is strongly associated with macrolide consumption.[21] Lu et al reported that 69% of patients with syphilis in China had a history of receiving macrolide treatment, although only 3.8% of patients with syphilis had been treated for syphilis.[8] Since azithromycin is not recommended for the treatment of syphilis, according to guidelines in China, the high level of macrolide resistance in *T. pallidum* in Xinjiang may be due to the extensive exposure of syphilis patients to macrolide drugs such as azithromycin for non-syphilis infections. This is supported by epidemiological evidence that macrolide exposure in the 3 months prior to syphilis diagnosis is associated with a 30% increase in resistance.[22]

A limitation of the current study is the lack of information on antibiotic treatment among syphilis patients; thus, the study was unable to provide direct evidence of the relationship between treatment failure and macrolide resistance.

In conclusion, the present study reported for the first time the molecular epidemiological profile of macrolide resistance in *T. pallidum* from patients with latent syphilis in Xinjiang, China. Macrolide resistance in *T. pallidum* should gain attention from clinicians in Xinjiang, China, to assist in decision making on the treatment options for syphilis in patients who are allergic to penicillin.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest in this work.

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