

# Gene subtype analysis of *Treponema pallidum* for drug resistance to azithromycin

YUECUI LI, JIN LI, WEIYUE HU, HONGXIA LUO, JING ZHOU, CHENGHANG LI and CHUNJIAO CHEN

Department of Infectious Diseases, The First People's Hospital of Yongkang, Yongkang, Zhejiang 321300, P.R. China

Received August 13, 2017; Accepted January 8, 2018

DOI: 10.3892/etm.2018.6241

**Abstract.** Azithromycin has been widely used for the treatment of *Treponema pallidum*. However, the drug resistance of *T. pallidum* for azithromycin is currently increasing. The aim of the present study was to analyze the association between gene subtypes of *T. pallidum* and drug resistance for azithromycin. The gene subtypes of *T. pallidum* were assayed by a polymerase chain reaction technique. Drug resistance of *T. pallidum* was analyzed using an antimicrobial susceptibility test. The results demonstrated that gene type *tpr* presented higher drug resistance compared with *arp* and *tp0548* gene types of *T. pallidum*. Gene type *tpr* was identified as eight gene subtypes (14a/f, 14e/f, 12e/f, 12d/f, 6d/f, 11d/f, 14j/f and 8d/f) among 324 cases. It was identified that 23S rRNA A2058G mutation was observed in gene subtypes 14a/f, 14e/f and 12e/f. A2059G mutation occurred in the gene subtypes 8d/f, 12d/f, 6d/f, 11d/f and 14j/f. The proportions of azithromycin-resistant genotypes harboring either the A2058G or the A2059G mutation among the *T. pallidum* strains were 34.2 and 65.8%, respectively. The antimicrobial susceptibility test demonstrated that A2059G mutations exhibited a higher drug resistance for azithromycin compared with A2058G mutations. In conclusion, these results indicate that azithromycin resistance in *T. pallidum* is associated with gene subtype, which may contribute to the treatment of *T. pallidum*.

## Introduction

Syphilis is a sexually transmitted disease caused by infection with the spirochete *Treponema pallidum* (1,2). Previous studies have indicated that the occurrence of syphilis may result in severe complications, including dermatological diseases, neurosyphilis and cardiovascular syphilis, which remain key causes of morbidity worldwide (3,4). In

addition, syphilis facilitates the infectivity and susceptibility to HIV infection in the clinic (5). Currently, the incidence of syphilis is increasing in multiple developed countries (6,7). Although various treatments have been explored, including antibiotic therapy and antiviral therapy, these regimens are unsatisfactory, particularly in specific populations (8,9). Therefore, early diagnosis and effective treatment of patients at the early stage of syphilis are essential for inhibition of *T. pallidum* infection.

Previous reports have indicated that antibiotic desensitization protocols may facilitate optimal and safe antibiotic therapy in the appropriate clinical setting for patients with *T. pallidum* infection (10). Alternative antibiotics treatments are widely used for patients with syphilis who are allergic to penicillin, including doxycycline, tetracycline, ceftriaxone, erythromycin and azithromycin (11,12). Clinical trials have indicated that the efficacy of oral administration of azithromycin (2.0 g) is equivalent to that of benzathine penicillin G for the treatment of early syphilis in patients without HIV infection (13). However, drug resistance of *T. pallidum* for azithromycin attenuates its therapeutic efficacy for patients with syphilis (14). Molecular analysis has indicated that the acidic repeat protein (*arp*) gene, *T. pallidum* repeat (*tpr*) gene and *tp0548* gene are associated with the drug resistance of *T. pallidum* (15,16). Therefore, it is critical to analyze the association between gene subtypes of *T. pallidum* and drug resistance of azithromycin.

The objectives of this study were to analyze the association between gene subtype of *T. pallidum* and drug resistance to azithromycin. In this study, *tpr*-positive specimens were analyzed for the presence of A2058G and A2059G mutations and the relative drug resistance to azithromycin was determined among *T. pallidum* strains.

## Materials and methods

**Sample collection and molecular strain typing.** A total of 132 blood samples were collected from female patients from the Yongkang region with primary syphilis in The First People's Hospital of Yongkang (Zhejiang, China) between May 2016 and June 2017. Patients with HIV or *T. pallidum* history were excluded from the present study. The protocols were approved by the Human Subjects Division of The First People's Hospital of Yongkang. All patients were required to provide written informed consent. *T. pallidum* strains were isolated from patient blood samples using sequencing-based typing or

---

*Correspondence to:* Dr Yuecui Li, Department of Infectious Diseases, The First People's Hospital of Yongkang, 599 Jinshan West Road, Yongkang, Zhejiang 321300, P.R. China  
E-mail: liyuecuihospital@sina.com

**Key words:** *Treponema pallidum*, gene subtype, azithromycin, drug resistance

enhanced Centers for Disease Control and Prevention typing as described previously (17). DNA samples were tested for *T. pallidum* using quantitative polymerase chain reaction (PCR) targeting the *polA* gene (18).

**DNA extraction.** *T. pallidum* DNA was isolated as previously described (19). Briefly, DNA was extracted from 200  $\mu$ l of whole blood and DNAzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to extract DNA from specimens, according to the manufacturer's protocols. Strain typing was classified based on the analysis of three DNA target regions: (1) Restriction fragment length polymorphism analysis of sequence differences in the *tpr* gene; (2) the number of 60 bp repeats in the *arp* gene; (3) sequence analysis of a short region of the *tp0548* gene (20).

**PCR methods.** The PCR assay for *T. pallidum* analyzed the gene target *polA* as described previously (21). The primers used were as follows: F: 5'-CGTGTGGTATCAACTATGG-3', R: 5'-TCAACCGTGTACTCAGTGC-3'. All PCR products were analyzed using an ABI9700 GeneAmp PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR was performed in a 25  $\mu$ l reaction volume with 0.1  $\mu$ g DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 0.5  $\mu$ M primers and 0.5  $\mu$ l Taq polymerase (Takara Bio, Inc., Otsu, Japan). PCR conditions were as follows: 96°C for 5 min, followed by 40 cycles at 95°C for 30 sec, 56°C for 56 sec and 72°C for 60 sec, followed by 72°C for 600 sec. PCR products were analyzed on a 1.5% agarose gel. Then, PCR products were visualized by staining with ethidium bromide and comparing with the molecular size markers of a 100- or 2,000-bp ladder (New England BioLabs, Inc., Ipswich, MA, USA).

**Detection of drug resistance to azithromycin.** The *polA* gene was amplified to detect resistance to azithromycin (22). Briefly, PCR amplification of the 23S rRNA gene of *T. pallidum* was treated by restriction enzyme digestion (*Mbo*II) as described previously (23,24). PCR were performed in a 50  $\mu$ l reaction volume with 0.2  $\mu$ g rRNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 0.5  $\mu$ M primers (5'-GTGCCAGCMGCCGCGG-3') and 1.0  $\mu$ l Taq polymerase. PCR conditions were as follows: 95°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 54°C for 57 sec and 72°C for 60 sec, followed by 72°C for 600 sec. The azithromycin resistance genotypes were analyzed by DNA sequencing of the PCR products after purification with a QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA, USA). *T. pallidum* DNA was also evaluated using restriction enzyme digestion for A2058G and A2059G mutations (*Mbo*II and *Bsa*I, respectively; New England BioLabs, Inc.) (25). The DNA sequences were obtained using the DNA analyzer 3730xl (Applied Biosystems; Thermo Fisher Scientific, Inc.) and analyzed using DNASTAR® software version 3.0 (DNASTAR Inc., Madison, WI, USA).

**Antimicrobial susceptibility testing.** All antibiotic disks (azithromycin) were purchased from Abtek Biologicals Ltd. (Liverpool, UK). An antibiogram was performed on Mueller-Hinton agar for 12 h at 37°C to determine the antimicrobial agents resistance profiles to azithromycin (10  $\mu$ g) or PBS (10  $\mu$ g). Antimicrobial susceptibility was performed

Table I. Overview of *tpr* gene subtypes of *Treponema pallidum*.

Gene type	A2058G	A2059G
14a/f	+	-
14e/f	+	-
12e/f	+	-
8d/f	-	+
12d/f	-	+
6d/f	-	+
11d/f	-	+
14j/f	-	+

Genotype was determined using sequencing-based typing or enhanced Centers for Disease Control and Prevention typing.

according to the European Committee on Antimicrobial Susceptibility guidelines (26).

**Statistical analysis.** Data are expressed as mean  $\pm$  standard deviation of triplicate experiments. All data were analyzed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Differences among groups were analyzed by one-way analysis of variance with Tukey's multiple comparisons test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Comparison of the gene types of *T. pallidum*.** DNA samples from 132 genital ulcer specimens were identified as *T. pallidum* positive, determined by PCR targeting the *polA* gene (Fig. 1A). Three gene types were observed among the 132 *T. pallidum* positive specimens (Fig. 1B). The restriction digestion assay indicated that 78 samples were *tpr* gene type, 24 were *arp* gene type and 30 were *tp0548* gene type. These results suggest that *tpr* is the most common gene type of *T. pallidum*.

**Analysis of *T. pallidum* drug resistance to azithromycin.** The antibiotic resistance of *T. pallidum* to azithromycin was analyzed in this study. As indicated in Fig. 2A, the restriction fragment length polymorphism analysis of the PCR amplicons from the representative samples (A2058G: 14a/f, 14e/f and 12e/f; A2059G: 8d/f, 12d/f, 6d/f, 11d/f, 14j/f.) revealed that 94 of the *T. pallidum* specimens were resistant to azithromycin. Gene type analysis indicated that the *tpr* gene type presented significantly higher drug resistance compared with the *arp* and *tp0548* gene types (Fig. 2B). These results suggest that *tpr* gene type may be associated with drug resistance of *T. pallidum* to azithromycin.

**Comparison of the gene subtypes *tpr* of *T. pallidum*.** The association between gene subtype *tpr* of *T. pallidum* and drug resistance was evaluated further. Eight subtypes of *tpr* (14a/f, 14e/f, 12e/f, 12d/f, 6d/f, 11d/f, 14j/f, 8d/f) were identified among the 132 cases (Table I). It was identified that 23S rRNA A2058G mutation was observed in gene subtypes 14a/f, 14e/f and 12e/f. A2059G mutation occurred in gene subtypes 8d/f, 12d/f, 6d/f, 11d/f and 14j/f. The proportion

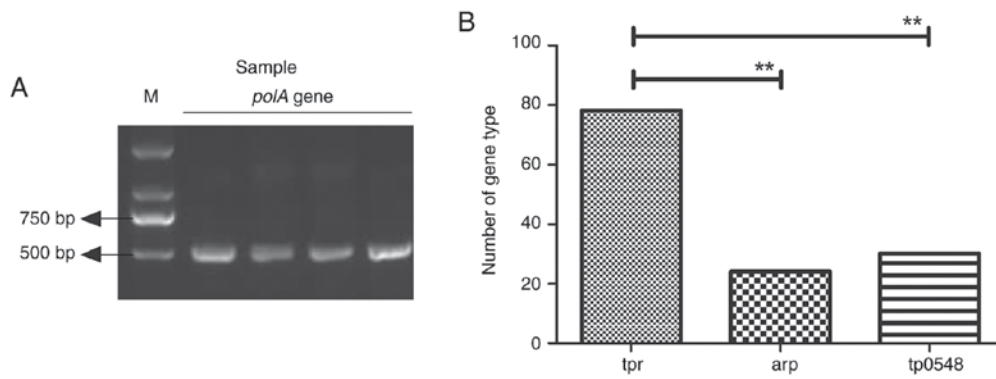


Figure 1. Comparison of the gene types of *Treponema pallidum*. (A) Identification of *T. pallidum* using PCR targeting the *polA* gene. (B) Gene type analysis in 132 *T. pallidum* specimens. \*\*P<0.01. M, marker.

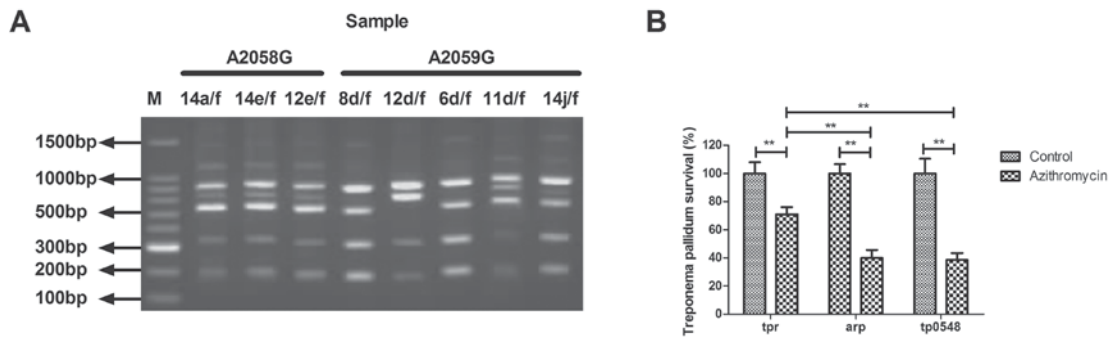


Figure 2. Analysis of *Treponema pallidum* drug resistance to azithromycin. (A) PCR products from representative samples used to analyze resistance to azithromycin in *T. pallidum*. (B) The *tpr* gene type presents higher drug resistance compared with the *arp* and *tp0548* gene types of *T. pallidum*. M, marker. \*\*P<0.01.

of azithromycin-resistant genotypes harboring either the A2058G or the A2059G mutation among *T. pallidum* was 65.8 and 34.2%, respectively (Fig. 3). These results indicate that *tpr* gene subtypes of *T. pallidum* may be associated with drug resistance to azithromycin.

**Analysis of drug resistance of A2058G and A2059G mutations in gene subtype *tpr* of *T. pallidum*.** The drug resistance of A2058G and A2059G mutations in gene subtype *tpr* of *T. pallidum* was investigated further. As indicated in Fig. 4A, A2059G mutation demonstrated a higher drug resistance for azithromycin compared with A2058G mutation, as determined by antimicrobial susceptibility test. The distribution of gene subtypes of *T. pallidum* with A2059G and A2058G mutation is presented in Fig. 4B and C. These results demonstrated that 14a/f of A2058G mutation and 12d/f of A2059G mutation frequently occurred in *tpr* of *T. pallidum*. These results indicate that A2058G and A2059G mutations in gene subtype *tpr* of *T. pallidum* are associated with drug resistance to azithromycin.

**Discussion**

Molecular subtyping for *T. pallidum* has previously been explored in the *tpr* gene and *arp* gene (27). A previous study also indicated that azithromycin treatment failures are associated with resistance in *T. pallidum* (28). However, the associations between molecular subtyping of *T. pallidum* and azithromycin resistance remain unclear. In the current study,

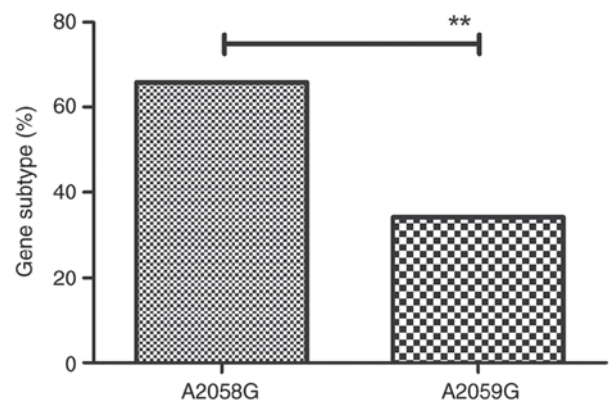


Figure 3. Proportion of azithromycin-resistant genotypes harboring either the A2058G or the A2059G mutation among *Treponema pallidum* samples. \*\*P<0.01.

the gene type of *T. pallidum* evaluated and azithromycin resistance was investigated in different gene types of *T. pallidum*. The results indicate that out of 132 samples, 78 were *tpr* gene type, 24 were *arp* gene type and 30 were *tp0548* gene type specimens of *T. pallidum*. The findings suggested that *tpr* gene type presented higher azithromycin resistance compared with *arp* and *tp0548* gene type specimens of *T. pallidum*.

Currently, azithromycin is widely used for the treatment of *T. pallidum* in patients that are allergic to penicillin (29). Although the recommended azithromycin treatment for syphilis

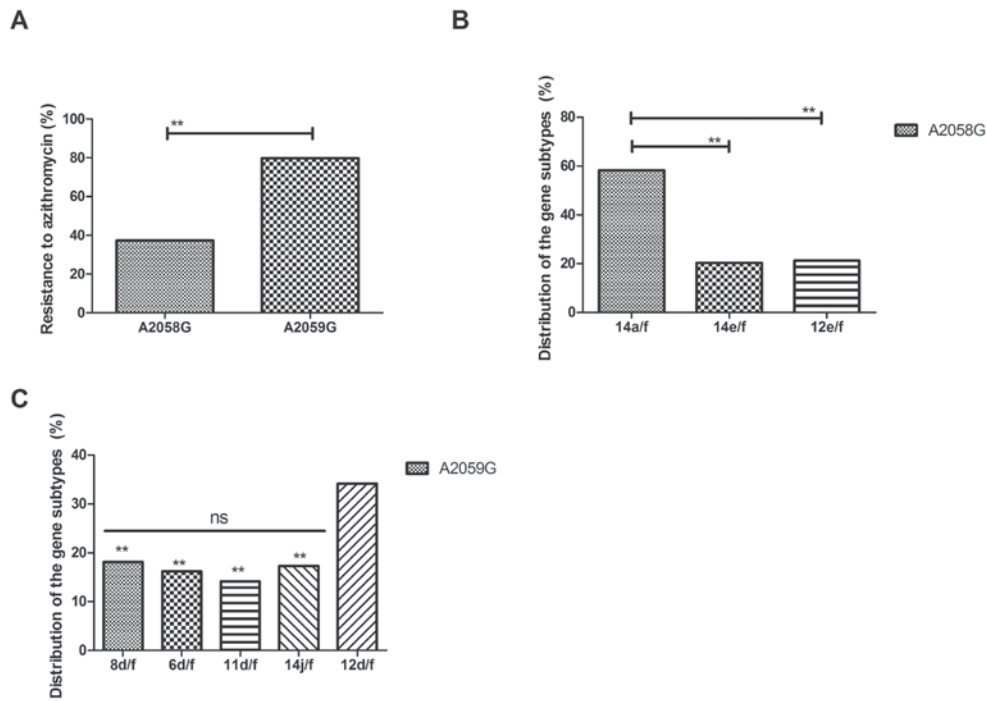


Figure 4. Analysis of drug resistance of A2058G and A2059G mutations in gene subtype *tpr* of *Treponema pallidum*. (A) A2059G mutation exhibits higher drug resistance for azithromycin compared with A2058G mutation. (B) Distribution of gene subtypes of *T. pallidum* with A2058G mutation. (C) Distribution of gene subtypes of *T. pallidum* with A2059G mutation. \*\* $P < 0.01$  vs. the 12 d/f group.

is effective, azithromycin resistance in *T. pallidum* has emerged and is increasing globally (30). It was observed that *T. pallidum* has developed azithromycin resistance to a varying extent (29). It has been identified that prevalence of azithromycin resistance is substantial in China and consequently that macrolides should not be used as a treatment option for early or incubating syphilis in China (31). The results of the current study identified three gene types, *tpr*, *arp* and *tp0548*, in 132 *T. pallidum* positive specimens from the Yongkang region. Reports have also indicated that restriction fragment length polymorphisms are associated with the drug resistance of *T. pallidum* (32,33). In the current study, it was identified that 94 specimens of *T. pallidum* presented restriction fragment length polymorphisms, which were associated with drug resistance of *T. pallidum* for azithromycin. Notably, gene type analysis suggested that the *tpr* gene type presents higher drug resistance compared with the *arp* and *tp0548* gene types of *T. pallidum*, which may be a potential target for addressing drug resistance to azithromycin.

A previous study indicated that *tpr* genes in *T. pallidum* are likely to be relevant to the pathogenesis of syphilis and drug resistance (34). A previous report described A2058G and A2059G mutations, which are associated with syphilis drug resistance to azithromycin (35). In the present study, the drug resistance to azithromycin of A2058G and A2059G mutations in *tpr* gene types of *T. pallidum* were compared. It was indicated that A2059G mutation demonstrated higher drug resistance for azithromycin compared with A2058G mutation. The findings also revealed that the gene subtypes of *T. pallidum* 14a/f of A2058G mutation and 12d/f of A2059G frequently occurred in *tpr* of *T. pallidum*, which may be a potential target for the treatment of syphilis. A limitation of the present study was that it did not explore the clinical treatment of azithromycin for patients with *T. pallidum*.

In conclusion, the current study identified that 12d/f *tpr* gene type of *T. pallidum* is the most common gene type for drug resistance to azithromycin. The findings suggest that A2059G mutation is associated with azithromycin resistance. Further investigation is required into the molecular mechanism of drug resistance of *T. pallidum*.

#### Acknowledgements

Not applicable.

#### Funding

No funding was received.

#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Authors' contributions

YL designed and performed the experiments. JL, WH, HL, JZ, CL and CC analyzed the experimental data and constructed the figures. The final version of the manuscript has been read and approved by all authors.

#### Ethics approval and consent to participate

The study protocol was approved by the Human Subjects Division of The First People's Hospital of Yongkang (Zhejiang, China). All patients provided written informed consent.

## Consent for publication

All patients provided written informed consent for the publication of their data.

## Competing interests

The authors declare that they have no competing interests.

## References

- Stoltey JE and Cohen SE: Syphilis transmission: A review of the current evidence. *Sex Health* 12: 103-109, 2015.
- Bristow CC, Larson E, Javanbakht M, Huang E, Causer L and Klausner JD: A review of recent advances in rapid point-of-care tests for syphilis. *Sex Health* 12: 119-125, 2015.
- Hawkes SJ, Gomez GB and Broutet N: Early antenatal care: Does it make a difference to outcomes of pregnancy associated with syphilis? A systematic review and meta-analysis. *PLoS One* 8: e56713, 2013.
- Gomez GB, Kamb ML, Newman LM, Mark J, Broutet N and Hawkes SJ: Untreated maternal syphilis and adverse outcomes of pregnancy: A systematic review and meta-analysis. *Bull World Health Organ* 91: 217-226, 2013.
- Campos LN, Guimaraes MD, Carmo RA, Melo AP, Oliveira HN, Elkington K and McKinnon K: HIV, syphilis, and hepatitis B and C prevalence among patients with mental illness: A review of the literature. *Cad Saude Publica* 24 (Suppl 4): S607-S620, 2008.
- Naesens R, Vermeiren S, Van Schaeren J and Jeurissen A: False positive Lyme serology due to syphilis: Report of 6 cases and review of the literature. *Acta Clin Belg* 66: 58-59, 2011.
- Marinkovic Z and Dukic S: Historical and medical review of syphilis-afflicted army leaders, rulers and statesmen. *Med Pregl* 64: 423-427, 2011.
- Seppings L and Hamill M: A review of an early syphilis outbreak in West Berkshire and Reading 2014-2015. *Sex Transm Infect* 92: 364, 2016.
- Kenyon CR, Osbak K and Tsoumanis A: The Global Epidemiology of Syphilis in the Past Century-A systematic review based on antenatal syphilis prevalence. *PLoS Negl Trop Dis* 10: e0004711, 2016.
- Magpantay G, Cardile AP, Madar CS, Hsue G and Belnap C: Antibiotic desensitization therapy in secondary syphilis and Listeria infection: Case reports and review of desensitization therapy. *Hawaii Med J* 70: 266-268, 2011.
- Balaskas K, Spencer S and D'Souza Y: Peripapillary choroidal neovascularisation in the context of ocular syphilis is sensitive to combination antibiotic and corticosteroid treatment. *Int Ophthalmol* 33: 159-162, 2013.
- Kahn RH, Moseley KE, Johnson G and Farley TA: Potential for community-based screening, treatment, and antibiotic prophylaxis for syphilis prevention. *Sex Transm Dis* 27: 188-192, 2000.
- Hook EW III, Behets F, Van Damme K, Ravelomanana N, Leone P, Sena AC, Martin D, Langley C, McNeil L and Wolff M: A phase III equivalence trial of azithromycin versus benzathine penicillin for treatment of early syphilis. *J Infect Dis* 201: 1729-1735, 2010.
- Read P, Jeffreys N, Tagg K, Guy RJ, Gilbert GL and Donovan B: Azithromycin-resistant syphilis-causing strains in Sydney, Australia: Prevalence and risk factors. *J Clin Microbiol* 52: 2776-2781, 2014.
- Castro R, Prieto E, Aguas MJ, Manata MJ, Botas J and Pereira FM: Molecular subtyping of treponema pallidum subsp. pallidum in lisbon, portugal. *J Clin Microbiol* 47: 2510-2512, 2009.
- Flasarova M, Smajs D, Matejkova P, Woznicova V, Heroldova-Dvorakova M and Votava M: Molecular detection and subtyping of Treponema pallidum subsp. Pallidum in clinical specimens. *Epidemiol Mikrobiol Imunol* 55: 105-111, 2006 (In Czech).
- Mikalova L, Strouhal M, Cejkova D, Zobanikova M, Pospisilova P, Norris SJ, Sodergren E, Weinstock GM and Smajs D: Genome analysis of Treponema pallidum subsp. Pallidum and subsp. pertenue strains: Most of the genetic differences are localized in six regions. *PLoS One* 5: e15713, 2010.
- Chen CY, Chi KH, George RW, Cox DL, Srivastava A, Rui Silva M, Carneiro F, Lauwers GY and Ballard RC: Diagnosis of gastric syphilis by direct immunofluorescence staining and real-time PCR testing. *J Clin Microbiol* 44: 3452-3456, 2006.
- Castro R, Aguas MJ, Batista T, Araujo C, Mansinho K and Pereira Fda L: Detection of Treponema pallidum Sp. Pallidum DNA in Cerebrospinal Fluid (CSF) by Two PCR Techniques. *J Clin Lab Anal* 30: 628-632, 2016.
- Kondratiev NV, Alfimova MV and Golimbet VE: A search of target regions for association studies between DNA methylation and cognitive impairment in schizophrenia. *Zh Nevrol Psikhiatr Im S S Korsakova* 117: 72-75, 2017 (In Russian).
- Pillay A, Liu H, Ebrahim S, Chen CY, Lai W, Fehler G, Ballard RC, Steiner B, Sturm AW and Morse SA: Molecular typing of Treponema pallidum in South Africa: Cross-sectional studies. *J Clin Microbiol* 40: 256-258, 2002.
- Liu H, Rodes B, Chen CY and Steiner B: New tests for syphilis: Rational design of a PCR method for detection of Treponema pallidum in clinical specimens using unique regions of the DNA polymerase I gene. *J Clin Microbiol* 39: 1941-1946, 2001.
- Jia S, Li J, Park SR, Ryu Y, Park IH, Park JH, Hong SS, Kwon SW and Lee J: Combined application of dispersive liquid-liquid microextraction based on the solidification of floating organic droplets and charged aerosol detection for the simple and sensitive quantification of macrolide antibiotics in human urine. *J Pharm Biomed Anal* 86: 204-213, 2013.
- Haanpera M, Huovinen P and Jalava J: Detection and quantification of macrolide resistance mutations at positions 2058 and 2059 of the 23S rRNA gene by pyrosequencing. *Antimicrob Agents Chemother* 49: 457-460, 2005.
- Lukehart SA, Godornes C, Molini BJ, Sonnett P, Hopkins S, Mulcahy F, Engelman J, Mitchell SJ, Rompalo AM, Marra CM and Klausner JD: Macrolide resistance in Treponema pallidum in the United States and Ireland. *N Engl J Med* 351: 154-158, 2004.
- Kassim A, Omuse G, Premji Z and Revathi G: Comparison of clinical laboratory standards institute and european committee on antimicrobial susceptibility Testing guidelines for the interpretation of antibiotic susceptibility at a University teaching hospital in Nairobi, Kenya: A cross-sectional study. *Ann Clin Microbiol Antimicrob* 15: 21, 2016.
- Tian H, Li Z, Li Z, Hou J, Zheng R, Li F, Liu R, Liu B, Wang C and Zhang F: Molecular typing of Treponema pallidum: Identification of a new sequence of tp0548 gene in Shandong, China. *Sex Transm Dis* 41: 551, 2014.
- Prevalence of the 23S rRNA A2058G point mutation and molecular subtypes in Treponema pallidum in the United States, 2007 to 2009. A2058G Prevalence Workgroup 39: 794-798, 2012.
- Pandori MW, Gordones C, Castro L, Engelman J, Siedner M, Lukehart S and Klausner J: Detection of azithromycin resistance in Treponema pallidum by real-time PCR. *Antimicrob Agents Chemother* 51: 3425-3430, 2007.
- Katz KA and Klausner JD: Azithromycin resistance in Treponema pallidum. *Curr Opin Infect Dis* 21: 83-91, 2008.
- Chen XS, Yin YP, Wei WH, Wang HC, Peng RR, Zheng HP, Zhang JP, Zhu BY, Liu QZ and Huang SJ: High prevalence of azithromycin resistance to Treponema pallidum in geographically different areas in China. *Clin Microbiol Infect* 19: 975-979, 2013.
- Chen CY, Chi KH, Pillay A, Nachamkin E, Su JR and Ballard RC: Detection of the A2058G and A2059G 23S rRNA gene point mutations associated with azithromycin resistance in Treponema pallidum by use of a TaqMan real-time multiplex PCR assay. *J Clin Microbiol* 51: 908-913, 2013.
- Wu BR, Yang CJ, Tsai MS, Lee KY, Lee NY, Huang WC, Wu H, Lee CH, Chen TC, Ko WC, *et al*: Multicentre surveillance of prevalence of the 23S rRNA A2058G and A2059G point mutations and molecular subtypes of Treponema pallidum in Taiwan, 2009-2013. *Clin Microbiol Infect* 20: 802-807, 2014.
- Giacani L, Molini B, Godornes C, Barrett L, Van Voorhis W, Centurion-Lara A and Lukehart SA: Quantitative analysis of tpr gene expression in Treponema pallidum isolates: Differences among isolates and correlation with T-cell responsiveness in experimental syphilis. *Infect Immun* 75: 104-112, 2007.
- Matejkova P, Flasarova M, Zakoucka H, Borek M, Kremenova S, Arenberger P, Woznicova V, Weinstock GM and Smajs D: Macrolide treatment failure in a case of secondary syphilis: A novel A2059G mutation in the 23S rRNA gene of Treponema pallidum subsp. pallidum. *J Med Microbiol* 58: 832-836, 2009.