MIXED INFECTION AND TWO DIFFERENT RESISTANCE PROFILES OF *TREPONEMA PALLIDUM* TO MACROLIDES IDENTIFIED IN SOME CLINICAL SPECIMENS OF THE SAME PATIENT

INFECÇÃO MISTA E RESISTÊNCIA DIFERENTE A MACROLÍDEOS EM TREPONEMA PALLIDUM IDENTIFICADAS POR VARIADAS ESPÉCIMES CLÍNICOS EM UM MESMO PACIENTE

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ABSTRACT
Syphilis represents a global public health problem. The resistance of *Treponema pallidum* to macrolides is related to the mutation in the *23S rRNA* gene (A2058G). We reported a case of secondary syphilis in a 52-year-old man presenting two profiles: the first one of susceptibility, and the other one of resistance, when we analyzed the *23S rRNA* gene sequence from two different clinical specimens of the same infectious episode. DNA from *T. pallidum* from skin biopsy presented resistance profile, whereas *T. pallidum* DNA from blood presented a profile of susceptibility to macrolides. These results suggest it was mixed infection or reinfection.
Keywords: syphilis; drug resistance; DNA sequencing.

RESUMO
A sífilis representa um problema de saúde pública mundial. A resistência de *Treponema pallidum* aos macrolídeos está relacionada à mutação no gene *23S rRNA* (A2058G). Relatamos um caso de sífilis secundária, em um homem de 52 anos, com um perfil de suscetibilidade e outro de resistência, ao analisarmos a sequência do gene *23S rRNA* de dois espécimes clínicos diferentes, do mesmo episódio infeccioso. A amostra de DNA de *T. pallidum* proveniente de raspado dérmico da lesão apresentou um perfil de resistência, enquanto aquele que derivou de sangue apresentou perfil de suscetibilidade aos macrolídeos. Esses resultados sugerem tratar-se de infecção mista ou de reinfecção.
Palavras-chave: sífilis; resistência; análise de sequência de DNA.

INTRODUCTION

The reemergence of syphilis in recent decades represents an important public health problem in developed and developing countries[1-5]. Syphilis can facilitate the Human Immunodeficiency Virus (HIV) transmission and increase the risk of adverse pregnancy outcome, besides the substantial social impact. The global syphilis incidence estimation for 2012 was 5.6 million cases. In Brazil, the primary and secondary syphilis in the population between the age group 15–49 years in 2010 was of 843,300 cases[4,6-9]. From 2005 to 2012, 57,700 prenatal syphilis cases were reported, most of which in the Southeast region[3,8-9].

A dose of 2.4 million units of penicillin G benzathine administered intramuscularly is the drug of choice for the syphilis treatment[10-12]. However, the intermittent lack of penicillin in the Brazilian market and the number of patients with reported allergies to penicillin[13] require other processes of treatment. The relatively low toxicity and strong macrolides bacteriostatic effect are the reasons for the use of erythromycin and azithromycin in the treatment of syphilis[11-14].

Unlike penicillin, the treatment of syphilis with macrolides represents risk of failure due to the resistance of *T. pallidum* subspecies *pallidum* to these antibiotics. The primary mutation in *T. pallidum* macrolide resistance is related to A2058G, as it occurs at the coding gene for 23S ribosomal RNA subunit (*23S rRNA*). Macrolide-resistant *T. pallidum* isolates represent a major challenge to public health in both developed and developing countries, making monitoring of *T. pallidum* resistant to macrolides an integral part of the syphilis control programs[5,9,10].

CASE REPORT

A 52-year-old man was treated at the Souza Araújo Clinic, of Oswaldo Cruz Institute (IOC) of Oswaldo Cruz Foundation (Fiocruz), in Rio de Janeiro, Brazil — a reference outpatient clinic of the Ministry of Health for the treatment of Hansen's disease —, with persistent skin and mucosal efflorescence for more than three months. The patient also complained of generalized symptoms such as fever, malaise, adenomegaly and intense headache. His background included infection with *Neisseria gonorrhoeae* two years before and genital lesion one year before. The patient reported having had sexual intercourse with several partners in the last six months.

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The patient was afebrile and revealed a 5 mm genital exulceration and macular rash on the torso and upper limbs on physical examination (**Figure 1**). Serological tests showed the following results: Venereal Disease Research Laboratory (VDRL) 1:256, haemagglutination for *T. pallidum* (TPHA) reagent, fluorescent treponemal antibody absorption test (FTA-ABS) reagent and HIV non-reagent. The genital lesion was not tested on a dark field test.

The molecular diagnosis was performed by polymerase chain reaction (PCR) specific for the amplification of gene *tpp15*, and for *T. pallidum* subspecies *pallidum*. The four clinical samples collected confirmed the diagnosis, and two of them (blood and *swab*) amplified for the *23S rRNA* gene, which were sequenced. The triplicate confirmation was analyzed in the SeqScape Software, that generated the chromatograms shown in **Figures 2 and 3**.

MATERIALS AND METHODS

Clinical samples, total blood with anticoagulant, Vacuplast (Beicheng, Zhejiang, China), serum and lesion scraping were collected prior to treatment initiation. For *T. pallidum* resistance diagnosis and molecular detection, DNA was extracted using *QIAamp DNA mini Kit* (Qiagen, Hilden, Germany), according to the manufacturer's instructions. PCR for the molecular diagnosis of *T. pallidum* amplifies a 120 bp fragment of the *tpp15* gene[15]. This reaction was followed by agarose gel electrophoresis analysis. The molecular detection of resistance to macrolide was carried out from PCR, that amplifies a 628 bp sequence of the *23S rRNA* gene, followed by a direct PCR product sequencing. Isolates of *T. pallidum* resistant to macrolides exhibited the A2058G mutation, as described by Lukehart *et al.* [8].

ETHICAL CONSIDERATIONS

This study was approved by the Ethics Research Committee of Fiocruz, Brazil (Certificate of Presentation for Ethical Consideration — CAAE — no. 58752716.5.0000.5248).

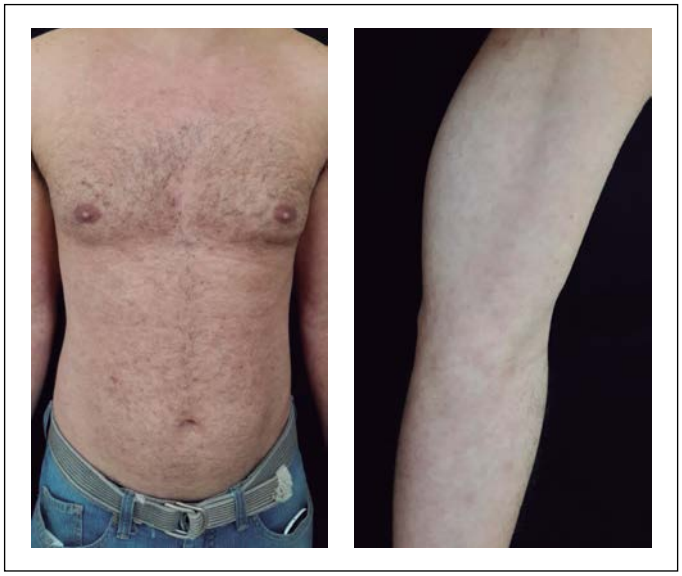

Figure 1 – Case patient presenting disseminated skin rash (syphilitic roseola).

RESULTS

Based on the clinical and serological findings, and evolution time, the patient was diagnosed as having secondary syphilis. Treatment with penicillin was administered intramuscularly in three doses of 2.4 million units. A monthly follow-up was carried out, and the patient reported improvement of symptoms, and at a physical examination four weeks after the last penicillin dose VDRL decreased (1:6).

Molecular detection of treponemal DNA may have identified the *T. pallidum* infection, as well as the macrolide resistance in clinical isolates. All clinical samples (biopsy, serum and total blood) collected from this patient were used for this detection. The PCR result, targeting the *tpp15* gene, was positive for all samples, thus confirming *T. pallidum* infection. Molecular detection of the *T. pallidum 23S rRNA* gene from the biopsy showed a transition from A to G at position 2058 (A2058G) related to the *T. pallidum* resistance to macrolides. This same DNA analysis obtained from total blood and serum showed a macrolide susceptibility profile for both samples, *i.e.*, without A2058G transition in the *23S rRNA* gene.

DISCUSSION

Although the patient was treated with penicillin, the discovery of at least one isolate of *T. pallidum* with a genotypic profile of macrolide resistance showed the importance of treating syphilis with the first antibiotic of choice[9,12]. Other drugs, such as azithromycin[8,11,16] and erythromycin, are recognized effective in the treatment of early syphilis in human beings. However, the increasing incidence of isolates of macrolide-resistant *T. pallidum* was reported in recent years[8,16]. It is believed that resistance to macrolides is due to the frequent use of these antibiotics for the treatment and prevention of a number of non-syphilitic infections[16].

Syphilis testing for macrolide-resistant treponemal are limited by the fact that *T. pallidum* cannot be grown *in vitro*, thus the routine test is restricted to the PCR amplification and sequencing of the *23S rRNA* gene from DNA of clinical samples of patients with syphilis[8]. The data on the occurrence of isolates resistant to macrolides are scarce, and we believe it could be explained because the syphilis molecular detection is not routinely performed.

This case proved to be even more interesting as it presents two resistance profiles, wild and mutant, in different samples, probably coming from the same episode of secondary syphilis. The *T. pallidum* isolate from genital lesion (*swab*) showed a genotypic profile in the *23S rRNA* gene with A2058G mutation, corresponding to an isolate macrolide-resistant, while *T. pallidum* DNA from total blood and serum did not show this transition. There are two possible explanations for this fact:

- the patient might have been infected with one sensible *T. pallidum* isolate and other macrolide-resistant isolate from the simultaneous intercourse with different partners, or at different moments, that formerly hosted the wild or mutant type in relation to drug sensitivity;
- the subject might have had sexual intercourse with an individual who also carried hetero-drug resistant isolates.

CONCLUSION

These data support the PCR testing utilization, since in this case it is possible to use different types of clinical samples for syphilis diagnosis and molecular identification. PCR can probably be used to diagnose syphilis in its different stages. Surveillance for resistance of *T. pallidum* to macrolides is also an important strategy for guiding the syphilis treatment recommendation, especially in periods of epidemic outbreaks and/or absence of the drug of first choice.

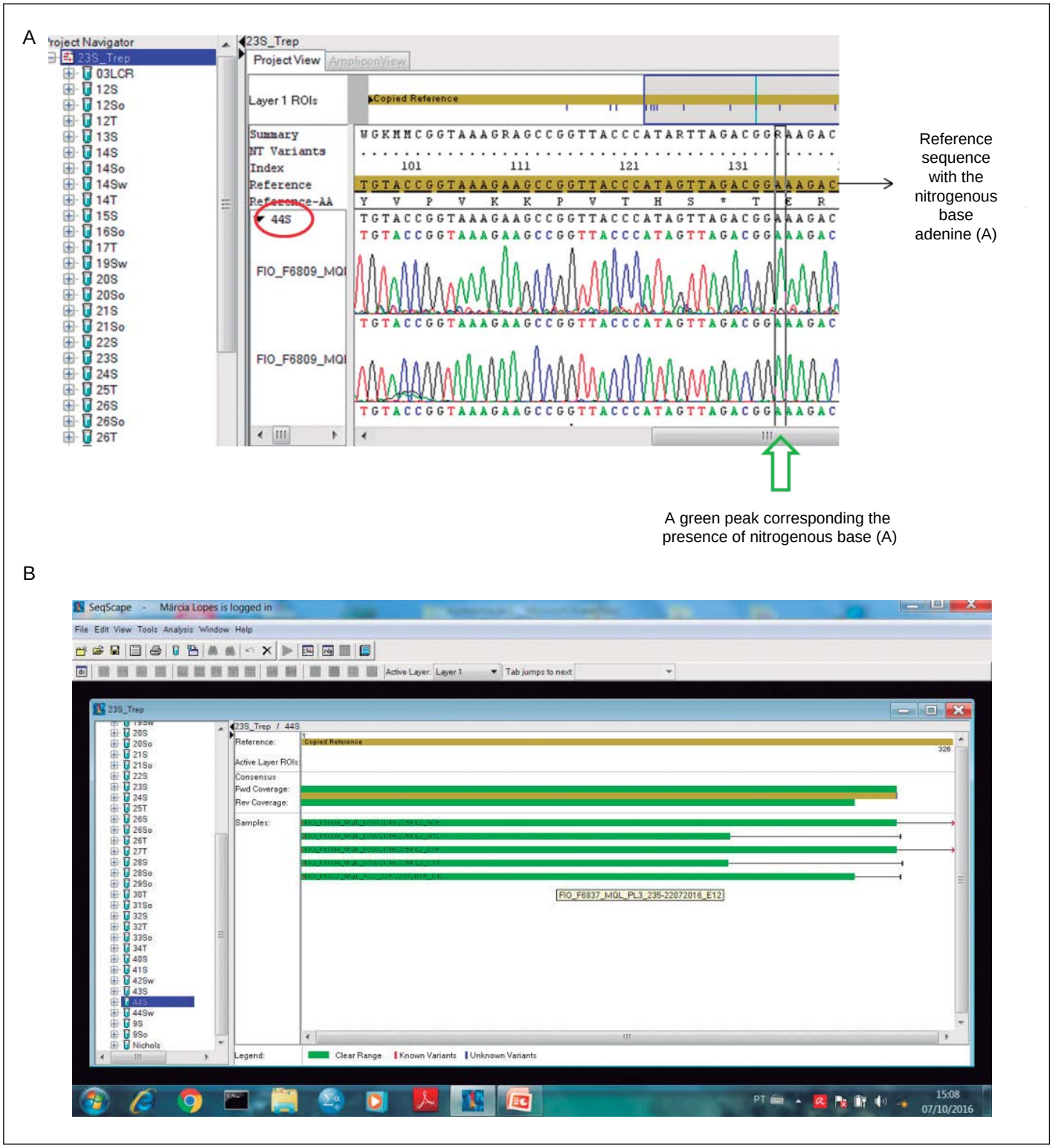

Figure 2 – Sample without mutation A2058G. (A) Visualization of the chromatogram within the SeqScape® program in which no nucleotide other than the reference sequence was found. (B) Image showing the sequences obtained from each initiator, as well as the region of exploitation of each of them (green). No base other than the reference sequence was identified in the sample.

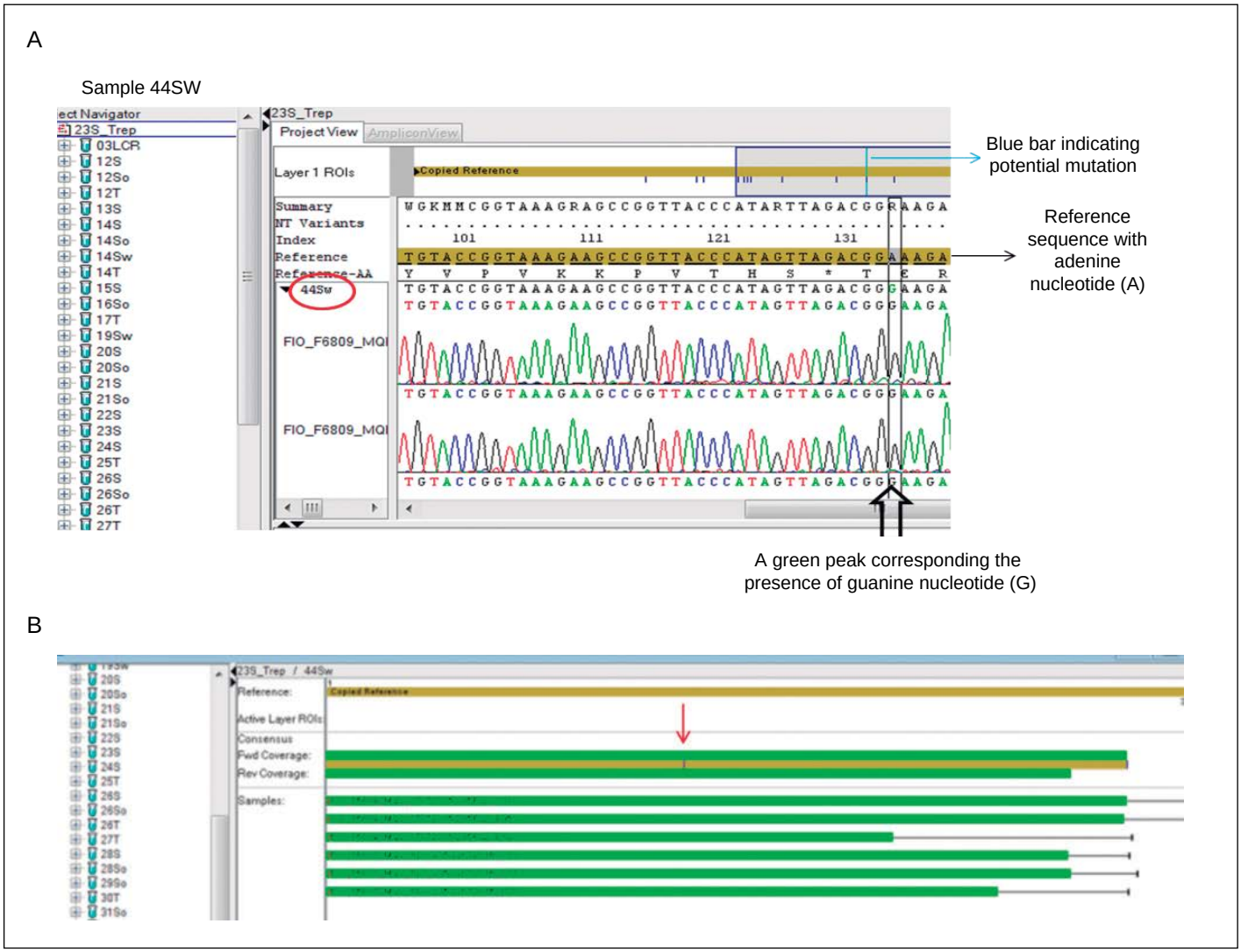

Figure 3 – Samples of the patient with mutation A2058G. (A) Visualization of the chromatogram within the SeqScape® program in which a nucleotide other than the reference sequence was found. (B) Image showing the sequences obtained from each initiator, as well as the region of exploitation of each of them (green). In this sample, a nucleotide (red arrow) distinct from the reference sequence was identified.

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Conflict of interests

The authors declare no conflict of interests.

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