Detection of *Treponema pallidum* subsp. *pallidum* from Skin Lesions, Serum, and CSF in an Infant with Congenital Syphilis after Clindamycin Treatment in Pregnancy

**Running title:** Detection of *T. p. pallidum* from Skin Lesions

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Abstract

We report a case of congenital syphilis in a newborn after clindamycin treatment in pregnancy. Using PCR detection of *tmpC* (TP0319) and DNA sequencing of genes TP0136 and TP0548, DNA sequences identical to *Treponema pallidum* subsp. *pallidum* strain SS14 were detected in the infant’s skin lesions, serum, and cerebrospinal fluid.

Key words: congenital syphilis, *T. p. pallidum* SS14, DNA sequencing, treatment of syphilis, clindamycin
A 2,200-g male infant was born by emergency cesarean section for placenta praevia after a 35-week gestation to a 20-year-old secundigravida mother; amniotic fluid was turbid.

The infant’s mother was diagnosed with secondary syphilis in screening in the 15th week of pregnancy. She had a macular rash and her serological findings were as follows: RPR 1:64, MHA-TP positive, FTA-ABS positive, ELISA IgG positive, ELISA IgM positive. Her husband had no clinical signs of the disease at that time, but his serology was positive: RPR 1:32, MHA-TP positive, FTA-ABS positive, ELISA IgG positive, IgM negative. The mother was given clindamycin therapy due to a history of allergy to penicillin, which was not tested by a penicillin skin allergy test. There was a significant decrease of RPR titer at the time of delivery; serological findings were as follows: RPR 1:16, MHA-TP positive, ELISA IgG positive, ELISA IgM slightly positive.

The infant developed respiratory distress shortly after birth. He required mechanical ventilation and was therefore admitted to the neonatal intensive care unit (NICU) with a presumptive diagnosis of congenital syphilis. On admission to the NICU, examination revealed a liver palpable at 3 cm below the right costal margin in the midclavicular line, and a spleen palpable 2 cm below the left costal margin. Abdominal gray-scale ultrasound showed no sonographic abnormalities of the hepatic parenchyma or the biliary structures. Long bone radiographs demonstrated osteochondritis of both upper and lower extremities, consistent with a diagnosis of congenital syphilis. Laboratory examination revealed a white blood cell count of $6.1 \times 10^9$ cells/l (with 28% neutrophils), a hemoglobin level of 100 g/l, and a platelet count of $11 \times 10^9$ cells/l. C-reactive protein was 138.1 mg/l.

The infant’s skin changes were the most significant with regard to the diagnosis of congenital syphilis. There were numerous vesicles with non-hemorrhagic content on his palms, fingers, soles, and crura. The vesicles on the infant’s fingers and soles were stripped open, baring shallow erosions.
There were suppurative margins in the areas adjacent to the vesicles and shallow erosions and a discrete macular rash without desquamation on the patient’s chest. No nasal discharge was presented.

Therapy with aqueous crystalline penicillin G (100,000 units/kg/day) was initiated and serological diagnostics and PCR of samples from skin lesions, cerebrospinal fluid (CSF), and serum were performed. Serological diagnostics included RPR, MHA-TP, FTA-ABS, ELISA IgG and IgM, and Western blot IgG and IgM.

Serological findings were as follows: RPR 1:16, MHA-TP positive, FTA-ABS positive, ELISA IgM positive, ELISA IgG positive, Western blot IgM positive (antibodies against TpN15, TpN17, and TpN47), and IgG positive. CSF serological analysis was uninterpretable due to contamination with blood and the small sample of CSF taken.

A nested PCR protocol amplifying the tmpC gene (TP0319, which encodes a putative membrane lipoprotein) was used to detect T. p. pallidum in the clinical samples (3). Molecular analysis revealed T. p. pallidum in samples from the infant’s skin lesions, serum, and CSF.

Sequencing of genes TP0136 and TP0548 in the PCR-positive samples revealed DNA sequences identical to T. p. pallidum strain SS14.

On postnatal day 8, mechanical ventilation was finished. On day 14, sepsis developed and a diagnosis of hepatic insufficiency and ascites was made. A peripheral venipuncture blood culture yielded Enterobacter cloaceae producing extended-spectrum ß-lactamase. This culture showed sensitivity to chloramphenicol, ciprofloxacin, colistin, amikacin, meropenem. The infant was started on ciprofloxacin therapy. On day 18, the patient’s status rapidly worsened due to hepatic insufficiency which ended in extreme hyperbilirubinemia (610 µmol/l); the infant died.

DNA from skin lesions, blood serum and CSF was isolated using QIAamp DNA Mini Kit (Qiagen) according to manufacturer’s instructions. PCR detection of T. p. pallidum was performed
as described previously (3). Briefly, primers oTP0319F (5´-CTGCTCATCGGCTGCTCTA-3´) and oTP0319R (5´-ACCACAGACTTCGACCCCATC-3´) were used to produce the first amplicon (773 bp) of nested PCR protocol detecting \textit{tmpC} gene (TP0319). During the second step, 451 bp PCR product was amplified using following primers: iTP0319F (5´-GAAGGTGGTGACTTTCGTCGT-3´) and iTP0319F (5´-CAAAACCCGCTTCAAAGAGA-3). A PCR reaction (25 µl) contained 0.125 µl of each 10mM dNTP, 2.5 µl of 10X PCR buffer, 0.25 µl of each primer (concentration 100 pmol/µl), variable volume of sterile distilled water (12-21 µl) and examined DNA isolate (1-10 µl). To this reaction, 0.05 µl of \textit{Taq} polymerase (5000 U/ml, New England BioLabs) was added. PCR reactions were amplified as follows: 94 °C (1 minute); 94 °C (30 seconds), 58 °C (30 seconds), 72 °C (1 minute), 30 cycles; 72 °C (10 minutes). Second step of PCR was performed identically with the following exceptions: 1µl of the PCR reaction containing the 773 bp amplicon was used as a template and 40 amplification cycles were applied. Final amplicons were analyzed using 2% agarose gel. Chromosomal DNA of \textit{T. p. pallidum} comprising TP0136 and TP0548 genes was PCR amplified using following primers: TP0136F (5´-AGTGTCTTCCTCGTCCGTTC-3´) and TP0136R (5´-CACGTGGTGGTGTTCAAACTT-3´) resulting in 1207 bp PCR product and TP0548F (5´-GCGGTCCCTATGATATCGTGT-3´) and TP0548R (5´-GAGCCACTTCAAGCCTACCTAG-3´) resulting in 1066 bp amplicon. PCR reactions were set up as described above and cycling conditions were as follows: 94 °C (1 minute); 94 °C (30 seconds), 55 °C (30 seconds), 72 °C (2 minutes), 30 cycles; 72 °C (10 minutes). Resulting PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and subjected to dideoxyn terminator sequencing using amplification primers and additional internal primers. Sequence analysis was performed in DNASTAR software (Lasergene).
The number of treponemal DNA copies (inferred from the maximal dilution of PCR-positive samples) was identical in serum and CSF and came to approximately $10^6$ DNA molecules per 1 ml of undiluted serum and CSF samples.

Treponemal DNA in congenital syphilis can be detected in a number of clinical samples including serum, whole blood, amniotic fluid, paraffin-embedded placental tissue, bullous targetoid skin lesions, and CSF (4, 7, 9, 11). Detection of treponemal DNA in whole blood and serum in patients with congenital syphilis appears to be more reliable than in adult patients (7, 9). This is probably due to the relatively higher concentration of treponemes in the blood of these patients. In our case, the number of treponemal DNA copies emphasizes the fact that in early congenital syphilis the concentration of treponemes in serum and CSF is similar and notably higher than in adults (3). These findings are consistent with the study by Michelow et al. in which central nervous system involvement in infants with congenital syphilis was best predicted by IgM immunoblotting of serum or a PCR assay of serum or blood (7).

Skin lesions either in the form of a typical vesiculobullous eruption (especially over the palms of the hands and the soles of the feet) or a maculopapular skin rash over the body are common presentations of early congenital syphilis and have been described in several studies (5, 6, 11). In a study of premature infants with congenital syphilis, 62% had unusual desquamation over palms and soles (6). Bone changes, hepatosplenomegaly, respiratory distress, cerebrospinal fluid abnormalities, and jaundice are the other major manifestations of the disease in premature infants (6). Moreover, syphilis is highly probable in all infants with a serum quantitative nontreponemal serologic titer that is fourfold greater than the mother's titer (1). In the case presented here, the infant’s serum RPR titer (1:16) was identical to the mother’s RPR in serum samples that were taken immediately after
delivery. There was a fourfold decrease of the initial mother’s RPR titer after treatment. Despite the
decrease, the infant was infected because of inadequate maternal treatment.

Parenteral penicillin G is the only therapy with documented efficacy for syphilis during
pregnancy. In experiment on rabbits, single intramuscular doses of clindamycin (15 or 40 mg/kg) did
not decrease treponemal counts significantly, but single injections of penicillin (10,000 units/kg)
reduced treponemal counts by more than 250-fold. Multiple intramuscular injections of clindamycin
reduced counts by 5- to 7-fold, whereas multiple doses of penicillin decreased treponeme counts by
greater than 300-fold. Despite partially crossing the placenta, clindamycin is far less effective than
penicillin in treating syphilitic lesions (2, 8). Pregnant women with syphilis who report a penicillin
allergy should be desensitized and treated with penicillin. Skin testing may be useful in pregnant
women to establish whether a penicillin allergy exists (1).

In conclusion, this report documents the importance of treating syphilis during pregnancy with
penicillin and endorses the use of molecular techniques to identify *T. p. pallidum* in clinical samples
to diagnose early congenital syphilis.

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FIGURE 1. The erosions on the infant’s soles were swabbed and DNA sequences identical to *T. p. pallidum* strain SS14 were identified in the samples using PCR detection of *tmpC* (TP0319) and DNA sequencing of genes TP0136 and TP0548.