High frequency of Nichols-like strains and increased levels of macrolide resistance in *Treponema pallidum* in clinical samples from Buenos Aires, Argentina.

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**Supplementary Methods.** Protocol for PCR product purification with polyethylene glycol.

PCR products were purified using polyethylene glycol (PEG), according to an in-house protocol. Briefly, 1.1 µl of PEG solution (PEG 0.2 g/ml, NaCl 0.15 g/ml) were added to 1 µl of PCR product, mixed and incubated for 15 minutes at 37°C. Then, centrifuged at 15,000 g at room temperature for 15 minutes. After adding 3.8 µl of cool 80% ethanol per µl of PCR product, the mixture was incubated one minute at room temperature and the supernatant was discarded. The DNA was left to dry at 37°C for 30 minutes and finally suspended in 25 µl of molecular-grade water.
### Supplementary Table S1. Primers used for nested-PCR

| Locus       | External primers (5’-3’) | Coordinates | Length of PCR product (bp) | Internal primers (5’-3’) | Coordinates | Length of PCR product (bp) |
|-------------|--------------------------|-------------|---------------------------|--------------------------|-------------|----------------------------|
| human beta-actin | AGCGCAAGTACTCCGTGTG\(^a\) | -           | 107                       | -                        | -           | -                          |
|             | CCGACTCATCGTACTCCTGCTT\(^a\) | -           |                           | -                        | -           | -                          |
| TP0105      | TTCTGTGCTCAGTCTGGTC      | -           | 637                       | TGCACGTGTGCCAATGCTGGTC   | -           | 376                        |
|             | TGCAACCATCGTATCGAAA      | -           |                           | CACAGTGCTCIAACAGCGCTGACG | -           | -                          |
| TP0319      | CTGCCTCATGCGGCTGCTCTA    | -           | 773                       | GAAGGTTGCTGACTTCGCTG     | -           | 451                        |
|             | ACCACAGACTTCGACCCATC     | -           |                           | CAAAACGCCGCTTAAAGAGA     | -           | -                          |
| TP0136      | AACCGTTAGCGCCCAACAT      | 157804-157823 | 1789                      | AGTGCTTCTCTGCTCGTTC      | 158206-158225 | 1206                  |
|             | TCCACGCTCAGCGGAAATCTC    | 159570-159589 |                           | CAAGTTGTCGGTCTCAAACTT    | 159392-159411 | -                          |
| TP0548      | TGGGGCCAATACCGGGAAGA     | 593136-593155 | 1567                      | GCGGCTCTATGATCTGGT       | 593285-593305 | 1065                    |
|             | TACGGGCATTCTCAGGATAG     | 594683-594702 |                           | GAGCCACTTCAGCCCTACTG     | 594330-594349 | -                          |
| TP0705      | GGTCTATTAGCGACCTTCTCT    | 772663-772684 | 1181                      | TGGCGCTTATCGTGAATAG      | 772917-772938 | 803                      |
|             | GCTTGAAACGATACCGGATAC    | 773822-773843 |                           | TAATCTCGCCGCCTGGATAG     | 743700-773719 | -                          |
| 23S rDNA\(^c\) | CGAAGGGGAAGCGAGGTGTAG   | 234704-234723, 283149-283168 | 1666 and 1658           | GTACCCGCAACCCGACACAG     | 234768-234786 | 629                      |
|             | GCACGAACACCTTTTTAC       | 236350-236369 |                           | AGTCAAACGCCCACTC         | 235378-235396 | -                          |
|             | GAACCGTCCCTGAAAACCTCA    | 284787-284806 |                           | AGTCAAACGCCCACTC         | 235378-235396 | -                          |

\(^a\) According to Nichols genome (CP004010.2). Coordinates are only included for typing loci.

\(^b\) A single pair of primers was used for beta-actin amplification.

\(^c\) Both copies of 23S rDNA gene were amplified.