Supplementary 1. Detailed description of the Macrolide resistance assay.

Macrolide resistance assay

From each sample positive for *M. genitalium*, nucleic acid was extracted (400 uL input and 50 uL output) with MagDEA Dx SV 200 kit on a magLead 12gC robot (Bioservices, Stockholm, Sweden). For amplification of the *M. genitalium* 23S rRNA gene of region V, we used a 20 µl reaction of 1 x PerfectaTM qPCR FastMix™ Low Rox, (Quanta Biosciences, Gaithersburg, Maryland, USA), 0.125% (w/v) BSA (Ultrapure™,Ambion, Thermofisher Scientific, MA, USA), 0.2 µM of each forward (Mg 23S-1992F) and reverse primer (Mg 23S-2138R) (LGC Biosearch Technologies, Risskov, Denmark), 0.05 µM of each probe for wild type (FAM-ACGGAAAGACCCCGTGA-MGB), mutant with G at position 2071 (VIC-ACGGGAAGACCCCGT-MGB) and mutant with G at position 2072 VIC-ACGGAGAGACCCCGT-MGB) (Thermo Fisher Scientific, Stockholm, Sweden), and 5 µl of nucleic acid extraction from each sample (1). The PCR reaction was carried out in an automated thermal cycler (ABI 7500 Fast Real-time PCR) in standard mode: as follows; 20’’at 95°C and then 50 cycles of 3’’ at 95°C and 1’ at 60°C.

Purified DNA of *M. genitalium* (Amplirun® *M. genitalium* DNA control, Vircell, Granada, Spain) was included as a positive control and water was used as a negative control in each run. The sensitivity of the PCR was about 70 copies/PCR using the *M. genitalium* DNA control (Vircell, Granada, Spain). Plasmids with mutation A2071G and A2072G served as positive controls for detection of each of these mutations.

Reference:

1. Forslund O, Hjelm M, El-Ali R, Johnsson A, Bjartling C. Mycoplasma genitalium and Macrolide Resistance-associated Mutations in the Skåne Region of Southern Sweden 2015. Acta Derm Venereol. 2017;97(10):1235-8.