Appendix 1. Technical details of the THD fecal test (index test).

Participants collected and stored a stool sample using the THD fecal test equipment (THD Spa, Correggio, Reggio Emilia, Italy). The THD device implements the following steps: (i) collection of a fecal sample of about 300 mg, (ii) its dilution in phosphate buffer solution, (iii) treatment of the solution, (iv) collection of final upshot. In detail, the treatment of the solution eliminates real time polymerase chain reaction inhibiting substances, such as hemoglobin and its degradation products, polysaccharide complexes, heavy metals and proteins. Additionally, it removes large molecules, such as fibers. The treated solution may finally be taken from the collector and processed for DNA extraction. DNA was extracted from final upshot by using the phenol-chloroform procedure, which is largely acknowledged to constitute a reliable substrate for DNA analysis[1,2].

After this last step, real time polymerase chain reaction was performed in order to achieve two goals: (i) detection of the bacterial gene encoding the 23S ribosomal RNA subunit indicating Helicobacter pylori infection and (ii) assessment of bacterial gene point mutations indicating resistance to clarithromycin and levofloxacin.

Negative and positive controls were tissue and stool samples from a pool of Helicobacter pylori negative and positive people detected by both histology and urea breath test[3].

The real time polymerase chain reaction technique we used to detect point mutations conferring Helicobacter pylori resistance to clarithromycin has been previously described and extensively employed and validated[4-6]. We investigated the A2142C, A2142G and A2143G point mutations in the bacterium gene encoding for 23S rRNA subunit.

We assessed the point mutations (C261A, C261G, G271A, A272G, G271T and A270T) conferring resistance to levofloxacin in the A-subunit of DNA gyrase (gyrA) gene of Helicobacter pylori by investigating amino acid substitutions at position 87 (asparagine to lysine) and 91 (aspartic acid to glycine, aspartic acid to asparagine, and aspartic acid to tyrosine) of encoded amino acidic

sequence[7-9]. The real time polymerase chain reaction technique to detect mutations conferring levofloxacin resistance was performed according to Glocker and Kist[10]. The following step was the high-resolution melting curve analysis using the CFX96 Touch™ Real-Time PCR machine (BioRad)[11].

Appendix 2. Technical details of the ^{13}C-urea breath test (reference standard).

After overnight fasting, a baseline breath sample was collected, then a 10 ml solution containing 75 mg of ^{13}C-urea and 1.5 g of citric acid was administered in 200 ml of water solution. Another breath sample was obtained 30 minutes after solution administration and the test was considered positive if there was a difference between the baseline sample and the 30-minute sample that exceeded 4 parts per 1000 of ^{13}carbon dioxide (CO_2)[12,13]. The test methodology was standardized in a multicentre Italian study[14].

All breath samples were analyzed using a single gas isotope ratio mass spectrometer (Sercon Abca authomated breath 13C analyzer, Cheshire, UK).

Appendix 3. Technical details of amplification curves of *Helicobacter pylori* DNA sequences at real time polymerase chain reaction. A: negative control is characterized by the absence of amplification curve; B: positive control is characterized by the presence of amplification curve (green) above the cut-off red line; C: negative participant is characterized by the absence of amplification curve; D: positive participant is characterized by the presence of amplification curve (purple) above the cut-off red line.