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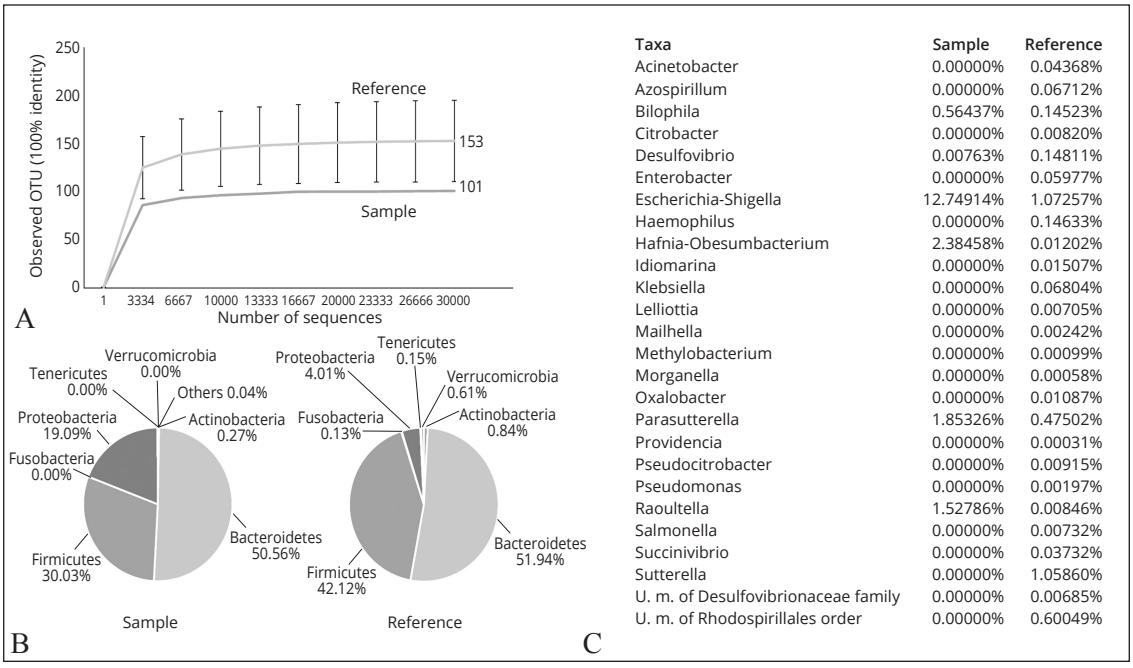
Can the analysis of the gut microbiota have a clinical application in real life?

The gut bacterial microbiota, in healthy human beings, is dominated mainly by genera belonging to the phyla Bacteroidetes and Firmicutes. Among healthy people, the relative percentage of each of these two dominant phyla can vary from 10% to 90%, but their combined percentage tends to be approximately 95% with the remaining 5% mainly constituted by genera belonging to the phyla Proteobacteria, Actinobacteria, Verrucomicrobia, Fusobacteria and Tenericutes.[1] Despite recent papers have highlighted the possible diagnostic role of gut microbiota analysis, like for instance in case of predicting a possible IBD flare or the response rate to low-FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, And Polyols) diet in IBS (Irritable Bowel Syndrome),[2, 3] this approach is really limited, or completely absent, from the diagnostic tool pipeline available for physician and/or nutritionists.[4, 5] In our department, we have currently adopted the gut microbiota analysis as a tool to collect more information about some of our patients. Here we describe the case, redacted according to CARE guidelines,[6] of a young male which gut symptoms have been explained, and then solved, by the results got by analyzing his stool microbiota. A 22-year-old male (BMI: 26.5) with an history of post-prandial headache, bloating, and rhinorrhea, more severe when eating milk-derivatives and/or fish, showed up at our hospital department, in early November 2022, requesting a gastroenterological visit aimed at understanding the reason for his increasingly frequent symptoms. During the visit the subject declared that these symptoms was appeared for the first time about four years ago but he was unable to recall events, such as illnesses or drugs, that could correlate temporally with the first episodes. The subject stated that in the past he had already tried to have a nutritional consultation, but the low-FODMAPs diet, that was proposed to him to reduce at least post-prandial bloating, did not have any effect. On the contrary, he argues that a diet rich in fruit and vegetables, both cooked and raw, did not provoke symptoms at all. Since the subject declared that both in the absence of milk derivatives and with a diet with a low protein content, post-prandial reactions such as headache, bloating and rhinorrhea did not occur, we requested allergy visit, lactose breath test, blood and urine analysis, and Hp (*Helicobacter pylori*) protein stool test. The allergy visit gave a completely negative result. The subject was also negative for lactose intolerance. The results of urine and blood analysis (complete blood count; protein electrophoresis; C-reactive protein; C3 and C4 complement proteins, IgG, IgA, IgM and IgE values, anti-peroxidase, anti-thyroglobulin, anti-transglutaminase and thyroid-stimulating hormone) revealed no abnormalities. The Hp stool antigens were also negative. Then, suspecting a gut dysbiosis, we proceeded analyzing the gut microbiota according to a methodology previously described.[7]

As shown in Figure 1, the subject presented a low bacterial richness and an increased relative value of Proteobacteria (19%) mainly at the expanse of Firmicutes with a reduction in the main butyrate-producers like *Agathobacter*, *Faecalibacterium* and *Roseburia* (data not shown). Noteworthy, among Proteobacteria *Escherichia/Shigella*, *Hafnia*, *Raoultella*, all histamine-producers,[8] showed a significant relative increase. Our assumption, derived by the subject symptoms and by his microbiota results, was therefore that he could have a SIBO mainly caused by the overgrowth of histamine-producers Proteobacteria. Our idea was that a protein-rich diet could stimulate these bacteria to convert histidine into histamine with such a great efficiency to overcome the quantity of the diamine oxidase (DAO), enzyme naturally present in the gut of any subject. To verify our hypothesis, we asked the subject to eat normally, including milk derivatives and fish, but taking pre-prandially a product containing enteric-coated DAO (Daosin, Stada), at the dose of two capsules before the main meals. The symptoms disappeared since the first day of treatment. To confirm that the pathological presence of the histamine-producers was mainly in the small intestine, we analyzed the urine content of indican and skatole[9] and performed the lactulose breath test.[10] The urine analysis revealed a higher value of indican (85 mg/dL; reference range: 1-20 mg/dL) and a normal value of skatole (<10 mg/dL; reference range: 1-20 mg/dL) suggesting a possible small intestine contamination. The very high basal value of H_2 (>40 ppm) during the examination confirmed the possibility of SIBO. We then treated orally the patient with rifaximin at 600 mg x 2/die for 14 days.[11] The symptoms disappeared, and the patients remained symptoms-free, with a free diet and without using the enteric-coated DAO, with a follow-up of about 160 days. After 60 days from the last dose of rifaximin we repeated the gut micro-



Taxa	Sample	Reference
Acinetobacter	0.00000%	0.04368%
Azospirillum	0.00000%	0.06712%
Bilophila	0.56437%	0.14523%
Citrobacter	0.00000%	0.00820%
Desulfovibrio	0.00763%	0.14811%
Enterobacter	0.00000%	0.05977%
Escherichia-Shigella	12.74914%	1.07257%
Haemophilus	0.00000%	0.14633%
Hafnia-Obesumbacterium	2.38458%	0.01202%
Idiomarina	0.00000%	0.01507%
Klebsiella	0.00000%	0.06804%
Lelliottia	0.00000%	0.00705%
Mailhella	0.00000%	0.00242%
Methylobacterium	0.00000%	0.00099%
Morganella	0.00000%	0.00058%
Oxalobacter	0.00000%	0.01087%
Parasutterella	1.85326%	0.47502%
Providencia	0.00000%	0.00031%
Pseudocitrobacter	0.00000%	0.00915%
Pseudomonas	0.00000%	0.00197%
Raoultella	1.52786%	0.00846%
Salmonella	0.00000%	0.00732%
Succinivibrio	0.00000%	0.03732%
Sutterella	0.00000%	1.05860%
U. m. of Desulfovibrionaceae family	0.00000%	0.00685%
U. m. of Rhodospirillales order	0.00000%	0.60049%

C

Figure 1.—Representation of the patient's gut microbiota at baseline: richness by rarefaction (A), phyla (B) and taxa of the Proteobacteria phylum (C).
The analyzed OTU (Operational Taxonomic Unit) number demonstrates a low biodiversity (A). According to reference values, an increased relative value of Proteobacteria (19%), mainly at the expanse of the phylum Firmicutes (about 30%) is observed (B). Some peculiar Proteobacteria taxa (C) show a significant relative increase: *Escherichia/Shigella* (about 12.75%), *Hafnia* (about 2.40%), *Raoultella* (1.53%). These taxa are described as gut histamine-producers.

biota analysis using the same procedures and the same laboratory. At the time of stool sampling, the subject had gained weight of about 2 kg. According to the new results (Figure 2), the rarefaction curve resulted a bit more reduced, by about 10%, in comparison with the earlier analysis (91 *versus* 101 OTUs) maybe as a direct consequence of the rifaximin treatment. Firmicutes (about 69%) become dominant, with *Agathobacter*, *Faecalibacterium* and *Roseburia* increased three-fold compared to reference (data not shown), at the expanse of Bacteroidetes (about 27%). Proteobacteria (less than 3%) went back to more typical values. As regards to histamine-producing Proteobacteria, *Escherichia* went back to a value 75% inferior to the reference, while *Hafnia* and *Raoultella* were undetectable. This case demonstrates that the results of the gut microbiota analysis clearly showed a picture of impairment due to the peculiar and exaggerated presence of these taxa. The controls we did to verify the information got by the microbiota analysis, that is the using an enteric-coated DAO enzyme as treatment, the checking of the indican and skatole content in urine and the performing of the lactulose breath test, have confirmed their goodness. This case surely does not mean that the subject has been definitively cured. Our follow-up lasted about four months and it is possible that the same situation could recur in the future as sometimes happens with SIBO. But this report demonstrates that, at least in some circumstances, the appropriate use of gut microbiota analysis does provide useful information about the clinical condition of a patient and can suggest a logical therapeutical approach. The information provided by the microbiota analysis are also more precise than those that we could have got by performing the lactulose breath-test, or by performing the more precise glucose breath-test, along with the urine analysis for determining indican and skatole. Indeed, without growing on agar medium the duodenal bacterial aspirate of the subject (procedure surely more invasive than sampling stool), the microbiota analysis has provided us with the precise taxonomical information about the taxa involved. Surely, the information got by using a product containing enteric-coated DAO enzyme, had provided us the information about the involvement of histamine in the patient's discomfort. Anyway, this check did not say nothing about the type of bacteria involved. Other than Proteobacteria, *Staphylococcus* spp., *E. faecalis* and *C. perfringens* are described to produce and release histamine in the gut as well.[11] Moreover, the second gut microbiota test allowed us to follow the bacterial patient picture, again without being invasive. As reported,[12] successful treatment of patients affected by histamine intolerance caused by the gut overgrowth of histamine-producer strains, demonstrates that the progressive reduction of Proteobacteria is accompanied by the progressive increase of Firmicutes and particularly

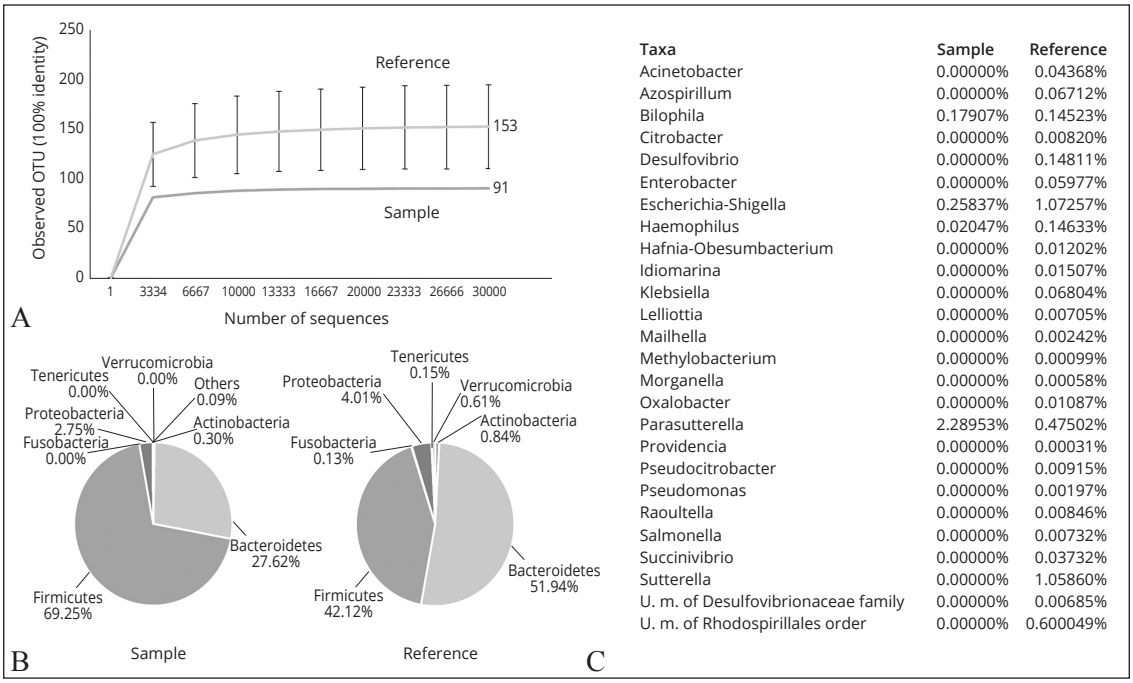


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Figure 2.—Representation of the patient's gut microbiota 60 days after rifaximin treatment: richness by rarefaction (A), phyla (B) and taxa of the Proteobacteria phylum (C).

The analyzed OTU (Operational Taxonomic Unit) number demonstrates a lower biodiversity not only in comparison with reference but also in comparison with the "baseline" richness (Figure 1). This further drop could be due to the antibiotic treatment (A). Firmicutes became dominant (about 69%) at the expanse of Bacteroidetes (now about 27%) and the main butyrate-producers (*Agathobacter*, *Faecalibacterium* and *Roseburia*) increased indeed three-fold compared to reference (see text). Proteobacteria (now less than 3%) have back to normal values (see reference value). *Escherichia/Shigella* is back to a value much inferior to reference, while *Hafnia* and *Raoultella* are now undetectable.

of those involved in butyrate production. The results obtained by the second analysis of the gut microbiota have shown exactly this phenomenon. In our opinion, the analysis of the gut microbiota should be part of the tools used for the benefit of the gastroenterological investigation.

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Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization r egarding t he m aterial d iscussed i n the manuscript.

Authors' contributions
Conceptualization, methodology, and formal analysis: all authors; writing (original draft preparation): Francesco Di Pierro, Viviana Gerardi, Stefania Piccirelli; writing (review and editing): Francesco Di Pierro, Nicola Zerbinati, Luigina Guasti, Alex Bertuccioli, Massimiliano Cazzaniga, Viviana Gerardi, Stefania Piccirelli, Daniele Salvi, Cecilia L. Pugliano, Paola Cesaro. All authors read and approved the final v ersion o f the manuscript.

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