SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

1. Stool samples collection and storage

Faecal samples (stools) from one confirmed COVID-19 patient (Sample A) and one healthy individual (Sample B) were obtained following written informed consent. Stool samples were kept at 4 °C until processing.

2. Medium preparation

Faecal samples were grown in NutriSelect™ Plus nutrient broth (n. 70122-500G, Sigma-Aldrich), fit for the growth of more fastidious bacteria. Following the protocol recommended by the supplier, the medium was prepared as follows: 25 g were dissolved in 1 L of double distilled water and filled into tubes. Tubes were sterilized by autoclaving at 121 °C for 15 minutes. All steps were conducted at temperature below 8°C, protected from direct light. Final composition of the medium was Peptone (15 g/L), Yeast extract (3.0 g/L), Sodium chloride (6.0 g/L). D(+)-Glucose (1.0 g/L), pH 7.5 at 25 °C.

3. Bacterial growing

Tubes/flasks with growing broth and bacteria were placed in an orbital shaker at 10 g at the optimal growing temperature of 37 °C and the liquid culture was left to grow progressively. Bacterial growth was monitored by measuring optical densities (OD) with a (spectro)photometer absorbance Microplate reader.

4. Nucleic acid extraction

Total nucleic acid extractions were performed using the NucliSens® easyMAG™ extraction system (bioMerieux, Marcyl'Étoile, France) according to the manufacturer's instructions, and as described in [1]. For our experiments, 250 µL of bacterial suspensions were subjected to extraction with an elution volume of 55 µL. The extracts were stored at −80 °C until use.

5. Determination of viral RNA load

Luminex technology (Life Technology, USA, see Dunbar[2] for an overview) was used to detect the viral RNA load in bacterial cultures. Aliquots of samples to be measured were always centrifuged at 1500 g for 10 minutes and supernatant was taken for the measurements.

The detection was performed by using NxTAG® CoV Extended Panel, a real-time reverse transcriptase PCR assay detecting three SARS-CoV-2 genes on the MAGPIX® NxTAG-enabled System MAGPIX instrument, and the AccuPlex™ SARS-CoV-2 Reference Material Kit (SeraCare) as reference standard with sequences from the SARS-CoV-2 genome.

Multiplex plates were in house produced and RNA tags were linked with the NaxPhot reagent before of the analysis.

Multiplex plates were transferred to the 37 °C pre-heated MAGPIX heater, and the signal was acquired by using the xPONENT and SYNCT software (Luminex Molecular Diagnostics). Each running batch handled up to 94 clinical specimens plus the positive and negative experiment controls. The total turnaround time was around 4 h. Luminex detection was reported in arbitrary units given by the formula (see Floridia[3]):

$$ARB = [\Sigma(SI*NF*COF)/ \Sigma (NF*COF)]$$

where SI: Signal intensity converted to Counts/s; NF: Noise Factor; COF: Correction Factor

6. Initial sample A and sample B processing

Both sample A and sample B were processed in parallel according to the following protocol:

- Homogenised faecal material was obtained by mixing it for 30 min in a pneumatic mixer (MiraclePaintSportDC-1-C, Minneapolis,MN,USA) with sterile distilled water (4 °C) in a proportion of 1.5 times the weight of a fraction of picked up sample.
- Homogenised faecal material was washed in 10 mL of $NaHCO_3$ 50 mmol (from Sigma Aldrich), pH 7.8, and centrifuged at 1,500 g for 10 minutes. Pellet was then re-suspended in 10 mL NaCl 10 mmol.
- The suspension was placed in a tube/flask with growing broth in an orbital shaker at 10 g at the optimal growing temperature of 37 °C. for one week.

7. Generation of sample $B_{(A+)}$ and C.

After one week of growing, 10 mL of sample A was centrifuged at 1,500 g for 10 minutes.
viral RNA load of supernatant was measured as described in point 5 and an aliquot corresponding to 180 ARB was inoculated to an aliquot of 5 mL of sample B, to generate sample $B_{(A+)}$.
Sample C was obtained by re-suspending in 10 mL of NaCl 10 mmol the pellet of sample A centrifugation.
In parallel with equivalent aliquots of sample A and sample B, sample $B_{(A+)}$ and sample C were placed in a tube/flask with growing broth in an orbital shaker at 10 g at the optimal growing temperature of 37 °C for 30 days. To measure RNA viral load, aliquots were collected at days 1, 2, 3, 7, 14, 21, and 30 following the date of inoculation (day 0).

8. Adding of antibiotics

At day 21 from inoculation, 18 aliquots of supernatant from sample $B_{(A+)}$ were taken and viral RNA load was measured for each of them as described in point 5. Then different antibiotics (metronidazole, clindamycin, lincomycin, piperacillin, tazobactam, vancomycin, amoxicillin, ampicillin, cefixime, ceftriaxone, meropenem, rifaximin, azithromycin, erythromycin, gentamicin, ciprofloxacin, colistin, levofloxacin, and teicoplanin) were added as follows.
The doses of used antibiotics were calculated according to the following formula

$$W=(1000/P)*V*C$$

where W is the weight of antibiotic and is an adimensional factor to be dissolved in V, the volume required; P, potency of the antibiotic base; C, final concentration of solution. The antibiotic doses were normalized to a weight value of 45.
Aliquots were homogenized for 30 min before the antibiotics treatments. The amount of added antibiotics was between 2 and 20 mg in the liquid phase aliquots (1 mL) according to the corresponding P. Antibiotics were placed in duplicates and incubated at 37 °C for 16–18 h both in disks and in solution. The disks were home made and used to verify the absence of bacterial resistance while the liquid was employed for Luminex measurements. In case of combined antibiotic doses were provided in 1:1 ratio. OD measurements were done before and after the treatment. After 3 days, viral RNA loads were measured in aliquots as described in point 5.
The used antibiotics were bought from different suppliers (Sigma Aldrich, Milan, Italy; GlaxoSmithKline, Milan, Italy; Ranbaxy Pharma, Milan, Italy).

9. Monitoring of bacterial growth and metabolic activity

In all samples and all aliquots of sample $B_{(A+)}$, bacterial growth and metabolic activity were analysed and monitored over time using SANIST Biotyper according to the method described by Cristoni et al.[4]

10. References

1. Yip CC, Sridhar S, Cheng A, et al. Evaluation of the commercially available LightMix® Modular E gene kit using clinical and proficiency testing specimens for SARS-CoV-2 detection. J Clin Virol. 2020 doi: https://doi.org/10.1016/j.jcv.2020.104476.
2. Dunbar SA. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. Clin Chim Acta. 2006. doi: https://10.1016/j.cccn.2005.06.023.
3. Floridia, M. & Cristoni, S. PROSAD: A powerful platform for instrument calibration and quantification. Rapid Commun. Mass Spectrom. 2014. doi: https://doi.org/10.1002/rcm.6808.
4. Cristoni S, Rossi Bernardi L, Larini M, et al. Predicting and preventing intestinal dysbiosis on the basis of pharmacological gut microbiota metabolism. Rapid Commun Mass Spectrom. 2019. doi: https://doi.org/10.1002/rcm.8461.