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The administration of *S. salivarius* K12 to children may reduce the rate of SARS-CoV-2 infection

The Coronavirus disease 2019 (COVID-19) pandemic, provoked by the worldwide spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused millions of infections and deaths worldwide since its emergence at the end of 2019. Unfortunately, natural immunity does not exist within the human population and no effective drug has been found thus far specifically for the disease. Incredibly, scientists from all over the world have developed some effective vaccines at an unprecedented pace.[1] Unfortunately, vaccine manufacturing and distribution are two important bottlenecks. For this reason, vaccination schedules are proceeding more slowly than they should and was perhaps expected. While waiting for the current vaccination campaigns to produce the herd effect desired by each individual country, COVID-19 research has produced new lines of scientific thought. For instance, recent evidence showed the possible relationship between the lung and oral microbiotas. Indeed, analysis of the bronchoalveolar lavage fluid (BALF) of COVID-19 patients revealed the presence of elevated levels of oral and upper respiratory commensal bacteria.[2] Anatomical and physiological considerations indicate that the oral cavity is the primary source of the lung microbiota community, acquired via aspiration and inhalation.[3] Indeed, the microbiota of the lungs overlaps with that found in the mouth. In humans, the prominent taxa in BALF samples include mainly *Streptococcus*, *Prevotella* and *Veillonella*, and these are indeed detected in concurrently collected oral samples. Recent studies have shown that the microbiota in the lungs contributes to immunological homeostasis and can potentially alter susceptibility to viral infection.[4] With respect to COVID-19, a particular abundance of *Prevotella* and *Veillonella* spp. in the lung microbiota composition has been observed in patients with SARS-CoV-2 pneumonia.[5] A report by Iebba *et al*.[6] (not yet peer-reviewed) profiled the oral microbiota of healthy controls and COVID-19-hospitalized patients, discovering the existence of four different bacterial consortia, which the authors named Species Interacting Groups (SIGs). In particular, SIG1 and SIG4, dominated mainly by *Prevotella* and *Veillonella* spp., were distinctive for COVID-19 pneumonia patients. Conversely, the same two taxa were not present in the SIGs distinctive of healthy controls, SIG2 and SIG3, which were instead characterized by the genus *Streptococcus*. Notably, the SIG2 consortium, showed, among the others, the presence of *S. salivarius*, an abundant representative of the normal oral consortium, also available as an oral probiotic. Regarding cytokines, the authors observed that SIG1 and SIG4 (those characterizing the COVID-19 oral microbiota) correlated with the presence of IL-6, a proinflammatory cytokine involved in the well-known "cytokine storm" characterizing a severe COVID-19 condition, while SIG2 and SIG3 (those characterizing the healthy control oral microbiota) did not. As stated by the authors, taken altogether these findings could suggest that some bacterial species from the beneficial SIGs may be used as local probiotics to restore the oral microbiota as a public intervention during the pandemic. Indeed, in a previous letter, the use of *S. salivarius* K12 was already proposed as an oral probiotic treatment to reduce the risk of SARS-CoV-2 infection and/or COVID-19.[7] In the first part (September-December 2020) of a still-ongoing randomized and controlled trial, aimed at evaluating the prophylactic role of oral probiotics in reducing bacterial and viral pharyngo-tonsillitis, 128 school-attending children within the Milan (Italy) area were enrolled and treated daily for 90 days (or received no treatment; control group), with *S. salivarius* K12. According to Table I, a nasal swab for detection of SARS-CoV-2 specific antigen was performed in 33 and in 46 children, respectively, in the treated and in the control groups. This difference was mainly due to dissimilarity with respect to the presence of typical symptoms, which were seen to a greater extent in the untreated children, potentially attributable to infection with SARS-CoV-2. Regardless of the frequency and intensity of the symptoms encountered, positivity to the antigen swab was detected only in 24 children within the control group. Out of these 24 children, seven had parents with COVID-19, four had brothers and/or sisters testing positive in the swab test for SARS-CoV-2 and 13 had classmates testing positive in the swab test for SARS-CoV-2. Adherence to therapy with strain K12 was reported by parents to be greater than 95% and no notable unwanted effects were reported during the trimester. This very preliminary report would seem to support the hypothesis of Iebba *et al*.[6] that the oral administration of oral-colonizing bacteria belonging to SIG2 or SIG3 could afford protection from SARS-CoV-2 infection and/or disease. Although only a randomized, double-blind, placebo-controlled trial can provide some certainty that a given medical approach reduces the risk

TABLE I.—*Features of the enrolled children; reason for the swab request; symptoms and diagnosis of SARS-CoV-2 infection.*

	Treated	Untreated	P
Total number	64	64	n. s.
Age of patients	7.7±3.2	8.2±3.0	n. s.
Male/female	38/26	28/36	n. s.
Total swabbed children	33/64	46/64	0.04
Due to clinical symptoms (A)	4/33	9/46	0.04
Due to close family contact (B)	12/33	11/46	n. s.
Due to positive classmate (C)	13/33	11/46	n. s.
A+B	2/33	7/46	n. s.
A+C	2/33	8/46	n. s.
Fever (>37.5<37.9)	4/8	23/24	0.01
Fever (>38.0)	0/8	1/24	n. s.
Pharyngodynia	2/8	24/24	0.01
Rhinitis	2/8	18/24	0.01
Headache	2/8	18/24	0.01
Gastroenteritis	2/8	9/24	n. s.
Myalgia	1/8	8/24	0.04
Cough	1/8	6/24	0.04
Dermatitis	0/8	3/24	n. s.
SARS-CoV-2	0/33	24/46	0.001
Age of positive		9.8±3.8	
Age of negative	7.7±3.2	7.3±1.9	n. s.
Males positive	0/38	10/28	0.001
Females positive	0/26	14/36	0.001

Age is expressed as years±standard deviation. All data were collected during the 90 days of administration of *S. salivarius* K12. Fisher's Exact test was used in the analysis of contingency tables. The Wilcoxon test was used to compare parameters of two different populations. JMP Pro v12 (SAS Institute Srl, Milan, Italy) was used to perform the analysis.
n. s.: non-significant.

of SARS-CoV-2 infection and/or disease, these results appear to be decidedly consistent with the hypotheses made regarding: 1) the close connection between the oral microbiota and lung microbiota; 2) the possibility that an oral microbiota not dominated by pathogenic or Gram-negative species favors the construction of a lung microbiota less likely to develop inflammatory responses to viruses; and 3) the idea that the administration of orally colonized bacteria belonging to the microbial consortia most frequently found in subjects not affected by COVID-19 is protective against infection.

The delay in vaccination campaigns and the health, psychological and financial difficulties suffered by a large part of the population are evident to all. This study is therefore an invitation to other researchers to verify in more controlled conditions the data that we have preliminarily observed and here anticipated, so that they could eventually be translated into clinical practice.

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Conflicts of interest.—Francesco Di Pierro has a financial interest with PharmExtracta S.p.A (Pontenure, Piacenza, Italy),

working in the scientific department where a finished form of strain K12 was developed.

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Clinical course and serial chest ultra-low-dose CT findings in a patient with COVID-19 treated with remdesivir

Since December 31st, 2019, a new bilateral pneumonia infection has been emerging in Wuhan Province, China. On January 7th, 2020, a new bat-derived beta coronavirus, SARS-Coronavirus-2 (SARS-CoV-2), was identified Coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2. The outbreak of COVID-19 in Italy in 2020 started in Lombardy, the Italian region bordering Canton Ticino (Switzerland), in February 2020. The first case of COVID-19 in Canton Ticino, Switzerland was identified on February 25th, 2020. On March 11th, 2020, the WHO declares a pandemic outbreak of coronavirus. We report a favorable clinical and radiological outcome in a young woman suffering from COVID-19 treated with remdesivir, that we were able to document using a sequence of high-quality, ultra-low-dose computer tomography (uldCT) of the chest.

On February 26th, 2020, a 47-year-old woman, without cardiopulmonary comorbidity presented during her holidays in Italy, with fever up to 39 °C, dry cough, and pharyngodynia. She received treatment with paracetamol following outpatient medical consultation. Five days later, she presented to our emergency department due to the dyspnea.

Upon admission the patient was in reduced general conditions, with body temperature 37 °C, sinus tachycardia 106 bpm, normal blood pressure (115/80 mmHg), tachypneic (30 acts/minute), and peripheral capillary oxygen saturation (SpO2) 95% with 2 L/min O_2. Pulmonary auscultation revealed diffuse bilateral basal crackles. C-reactive protein was 61 mg/L, leucocytes (5.1 $\times 10^9$/L). Nasal swab for influenza and respiratory syncytial virus (RSV), and urinary antigens for legionella and pneumococcus were negative. Bilateral infiltrates were observed on the chest X-ray (Figure 1A) and arterial blood gas analysis showed respiratory insufficiency (PaO_2/FiO_2 = 236 mmHg).

Suspecting a COVID-19, we performed a chest uldCT, which showed diffuse ground glass infiltrates (Figure 1B) suggesting severe acute respiratory syndrome (SARS). Nasal swab result confirmed the presence of SARS-CoV-2 (with rRT-PCR technique) on the same day.

A therapy with lopinavir/ritonavir (400/100 mg *b.i.d.*) was administered. We prescribed hydroxychloroquine (200 mg *b.i.d.*) as add on therapy based on preliminary clinical studies suggesting that chloroquine alone or in combination have some effect against SARS-CoV-2 [5]. After three days due to further respiratory deterioration, the patient was transferred to the intensive care unit where she was given high-flow oxygen therapy. Therefore, we changed the antiviral therapy to remdesivir (200 mg IV loading dose, then 100 mg IV daily × 9 additional days), obtained in a compassionate use program and administered for ten days.

A progressive favorable clinical course (Figure 2) well documented by two additional chest uldCT at day nine and 15 (Figure 1C, D) was observed.

In this patient presenting with fever, dyspnea and cough, the diagnosis of COVID-19 was obtained by

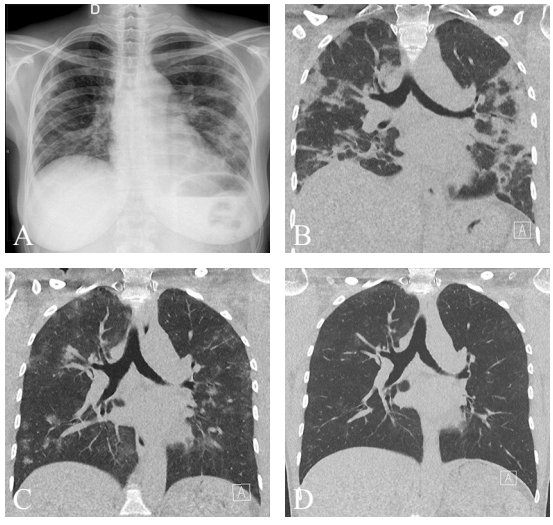

Figure 1.—Presence and progressive healing at day 1 (A), 2 (B), 9 (C) and 15 (D) of bilateral infiltrates with subpleural distribution.