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Efficacy of a Probiotic Consisting of *Lacticaseibacillus rhamnosus* PDV 1705, *Bifidobacterium bifidum* PDV 0903, *Bifidobacterium longum* subsp. *infantis* PDV 1911, and *Bifidobacterium longum* subsp. *longum* PDV 2301 in the Treatment of Hospitalized Patients with COVID-19: a Randomized Controlled Trial

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Abstract
The treatment of coronavirus disease (COVID-19) and COVID-19-associated diarrhea remains challenging. This study aimed to evaluate the efficacy of a multi-strain probiotic in the treatment of COVID-19. This was a randomized, controlled, single-center, open-label trial (NCT04854941). Inpatients with confirmed COVID-19 and pneumonia were randomly assigned to a group that received a multi-strain probiotic (PRO group) or to the control group (CON group). There were 99 and 101 patients in the PRO and CON groups, respectively. No significant differences in mortality, total duration of disease and hospital stay, incidence of intensive care unit admission, need for mechanical ventilation or oxygen support, liver injury development, and changes in inflammatory biomarker levels were observed between the PRO and CON groups among all included patients as well as among subgroups delineated based on age younger or older than 65 years, and subgroups with chronic cardiovascular diseases and diabetes. Diarrhea on admission was observed in 11.5% of patients; it resolved earlier in the PRO group than in the CON group (2 [1–4] vs. 4 [3–6] days; $p=0.049$). Hospital-acquired diarrhea developed less frequently in the PRO group than in the CON group among patients who received a single antibiotic (0% vs. 12.5%; $p=0.023$) unlike among those who received > 1 antibiotic (10.5% vs. 13.3%; $p=0.696$). The studied probiotic had no significant effect on mortality and changes in most biomarkers in COVID-19. However, it was effective in treating diarrhea associated with COVID-19 and in preventing hospital-acquired diarrhea in patients who received a single antibiotic.

Keywords COVID-19 · Probiotics · Diarrhea · Mortality · Liver

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory infection with systemic manifestations [1]. Despite intensive research, COVID-19 treatment remains an important challenge [2]. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host [3]. They have been reported to show a positive effect on acute respiratory infections by modulating immune responses [4].

Diarrhea is a manifestation of COVID-19, and has been reported in approximately 10% of COVID-19 patients [5]. COVID-19-associated diarrhea has distinct characteristics [6], and may even be the first manifestation of COVID-19 [7]. Although the exact mechanisms of the development of diarrhea in COVID-19 remain unknown, the following factors of its pathogenesis can be suggested. The viral E protein binds to proteins of the tight junctions of enterocytes, which leads to an increase in the permeability of the intestinal barrier, bacterial translocation, and gut inflammation. Moreover, the viral proteins E and Orf3a also disrupt the functioning of ion channels in the enterocyte. In addition,

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the pathogenesis of diarrhea in COVID-19 patients may also include intestinal damage during a cytokine storm, gut dysbiosis, and *Clostridioides difficile* superinfection [8]. A number of probiotics have been shown to be effective in the treatment of diarrhea of various etiologies [9–11].

Liver injury develops in an average of 20–25% of COVID-19 patients [12]. Several probiotics have been shown to be effective in the treatment of certain liver diseases [13].

Many experts have suggested the use of probiotics in combination with other drugs for the management of COVID-19 [14–21].

The effect of multi-strain probiotics on COVID-19 has been evaluated in three published retrospective studies [22–24]. Li et al. reported that the administration of a probiotic including *Bifidobacterium longum* subsp. *infantis*, *Bifidobacterium longum* subsp. *longum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bacillus cereus, Bacillus subtilis*, *Streptococcus thermophiles*, and *Enterococcus faecium* led to a slight decrease in hospitalization duration and accelerated the clearance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 [22]. Ceccarelli et al. showed that the use of a probiotic including *S. thermophilus* DSM 32,245, *Bifidobacterium lactis* DSM 32,246, *Bifidobacterium lactis* DSM 32,247, *L. acidophilus* DSM 32,241, *L. helveticus* DSM 32,242, *Lacticaseibacillus paracasei* DSM 32,243, *L. plantarum* DSM 32,244, and *L. brevis* DSM 27,961 reduced mortality in COVID-19 patients [23]. D'Ettorre et al. described the positive effects of the same probiotic on diarrhea associated with COVID-19 [24].

However, these findings have not been verified in prospective randomized studies. The aim of our study was to evaluate the effect of a multi-strain probiotic on mortality, disease course, respiratory function, diarrhea, and liver injury in COVID-19 patients in a randomized controlled trial.

Materials and Methods

This was a randomized, controlled, single-center, open-label trial. All patients signed an informed consent for study participation and the use of off-label drugs. The study was approved by the local ethics committee (Conclusion №. 34–20 of September 9, 2020) in accordance with the Declaration of Helsinki, and was registered at https://clinicaltrials.gov (NCT04854941). The research protocol can be accessed at this website as well. The study received no funding.

Patients

The study included patients with COVID-19 admitted to the Clinic of Internal Diseases of Sechenov University. COVID-19 was confirmed using polymerase chain reaction on nasopharyngeal and oropharyngeal swabs to detect SARS-CoV-2. The study was conducted from December 2020 to March 2021 and included participants in the age range from 18 to 75 years.

The exclusion criteria were as follows: age over 75 years or under 18 years, consumption of probiotics for 3 months prior to admission, presence or history of intolerance to probiotics or their components, refusal to participate and sign informed consent, pregnant or breastfeeding, cancer or mental illness, and severe renal (glomerular filtration rate less than 50 mL/min) or hepatic (equivalent to cirrhosis class B or C on the Child–Pugh scale) dysfunction at the time of admission.

Patients who prematurely discontinued the consumption of probiotics for reasons not related to the development of side effects were excluded from the study.

Intervention

The patients were randomized to the probiotics group (PRO group) or the control group (CON group). During the hospital stay, patients in the PRO group received the following probiotics three times a day for no more than 14 days: Florasan-D containing ~ 10^9 colony forming units (CFU) of *Lacticaseibacillus rhamnosus* PDV 1705, ~ 10^9 CFU of *Bifidobacterium bifidum* PDV 0903, ~ 10^9 CFU of *Bifidobacterium longum* subsp. *infantis* PDV 1911, and ~ 10^9 CFU of *Bifidobacterium longum* subsp. *longum* PDV 2301. The end point of the trial was day 14 of hospitalization or the day of the patient's discharge or death, whichever occurred earlier. The follow-up period lasted from the time of the inclusion until recovery or death.

Controls

The control group consisted of patients who did not receive probiotics.

Patients in both the groups also received dexamethasone and antiviral (favipiravir and/or riamilovir), antibacterial, anticoagulant (enoxaparin in most cases; rivaroxaban and dabigatran were used less frequently), and anticytokine (tocilizumab or/and olokizumab) drugs according to indications and contraindications (Table S1).

Outcomes

Death from any cause, duration of hospitalization, total duration of the disease, incidence of admission to intensive care unit, need for oxygen support or mechanical ventilation, and changes in the values of key biomarkers were considered as the main outcomes. The duration of diarrhea [an increase in the frequency of bowel movements (more than three times per day)], incidence of hospital-acquired diarrhea, progression of pre-existing liver injury, and onset of liver injury were considered as additional outcomes. Liver injury was determined

based on the presence of abnormalities in any of the main liver test findings (serum alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, and albumin levels).

The volume of the affected lungs was measured using chest computed tomography (CT); it included the sum of ground glass and consolidation volumes.

We performed per-protocol analysis as there was no final point to perform intention-to-treat analysis.

Statistics

Results are presented as median [interquartile range]. The groups were compared using the Mann–Whitney test for continuous data and chi-square test for categorical data. Wilcoxon test was used to assess changes in continuous biomarker values. Mortality was assessed using the Kaplan–Meier estimator and Cox's test. A p value ≤ 0.05 was considered as the criterion for significance. Statistical calculations were performed using STATISTICA 10 (TIBCO Software, Palo Alto, CA).

Results

Characteristics of the Included Patients by Groups

The study included 99 patients in the PRO group and 101 patients in the CON group (Fig. 1). COVID-19 pneumonia was confirmed in all patients using a chest CT scan. There was no significant difference in age, sex distribution, body mass index, body temperature at admission, symptoms of COVID-19, incidence of co-morbidities, and use of other drugs for the treatment of COVID-19 between the groups (Table 1; Table S1).

Main Outcomes

No significant differences in the total duration of disease, length of hospital stay, incidence of intensive care unit admission, need for mechanical ventilation or oxygen support (Table 1), and the changes in the volume of the affected lungs and serum levels of biomarkers of systemic inflammation [C-reactive protein (CRP), erythrocyte sedimentation rate, ferritin, fibrinogen, white blood cells, neutrophils, and lymphocytes], renal function (creatinine), and liver function (alanine aminotransferase, aspartate aminotransferase, albumin, and total bilirubin) were observed between the groups (Tables 2 and 3).

The patients in the PRO and CON groups had similar survival rates ($p = 0.491$) (Table 1; Fig. 2a). Two patients who stopped taking the probiotic prematurely also survived.

As the presence of cardiovascular diseases or diabetes is a risk factor for poor prognosis in COVID-19 [25], an analysis was performed for these subgroups of patients. The administration of the probiotics had no significant effects

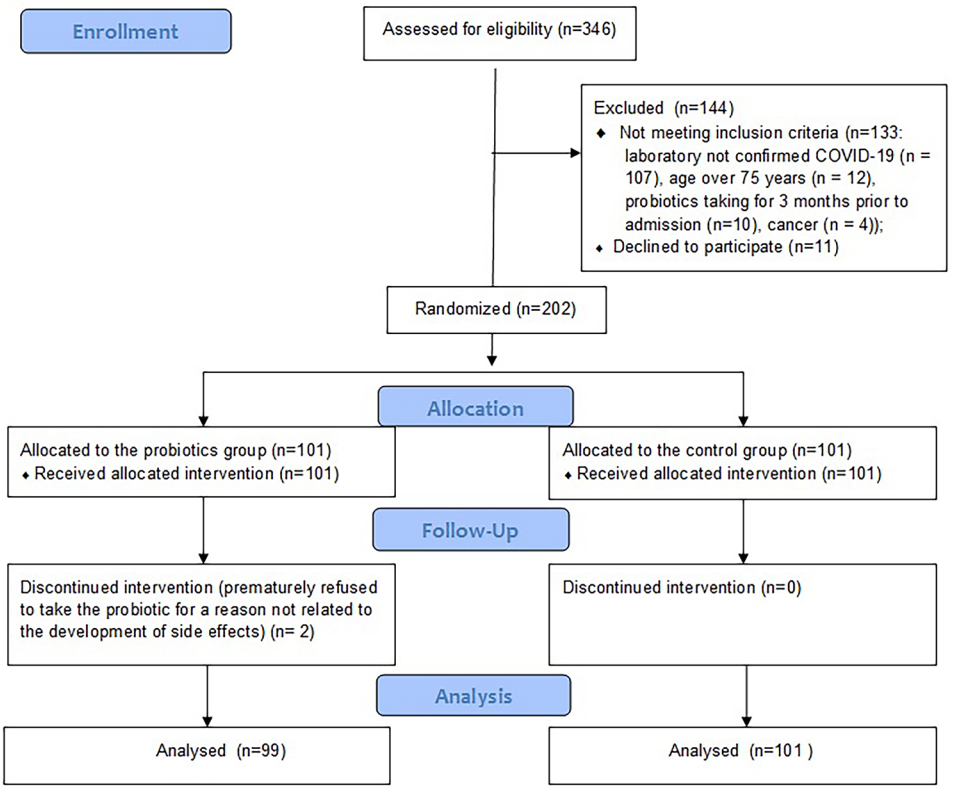

Fig. 1 CONSORT 2010 flow diagram

Table 1 Main characteristics and outcomes of patients who received probiotics (PRO group) and who did not (CON group)

	Group PRO ($n=99$)	Group CON ($n=101$)	p
Age, years	65 (59–71)	64 (54–70)	0.283
Male/female	44/55	48/53	0.662
Body temperature at admission, °C	37.3 (36.9–37.7)	37.2 (36.8–37.6)	0.657
Body mass index, kg/m^2	30.5 (27.4–35.3)	31.2 (27.1–33.5)	0.910
Time from symptom onset to inclusion, days	8 (6–12)	9 (7–11)	0.504
Length of hospital stay, days	11 (10–14)	11 (9–14)	0.440
Total duration of disease, days	20 (18–24)	21 (18–25)	0.471
Death, *n* (%)	4 (4.0%)	4 (4.0%)	0.491
Admission to intensive care unit, *n* (%)	5 (5.1%)	7 (6.9%)	0.576
The need for mechanical ventilation, *n* (%)	4 (4.0%)	5 (5.0%)	0.976
Oxygen support, *n* (%)	47 (47.5%)	44 (43.6%)	0.579
Duration of oxygen support, days	6 (2–11)	7 (1–11)	0.513
Patients with diarrhea on admission, *n* (%)	12 (12.1%)	11 (10.9%)	0.785
Duration of diarrhea on admission, days	2 (1–4)	4 (3–6)	0.049
Patients with hospital-acquired diarrhea, *n* (%)	4 (4.0%)	10 (9.9%)	0.109
Duration of hospital-acquired diarrhea, days	4 (4–6)	5 (3–6)	0.777

on the course of COVID-19 in the aforementioned patients, except for a tendency towards a decrease in mortality rate in patients with cardiovascular diseases (Table S2; Fig. 2b, c).

Probiotic administration did not exert significant effects on the course of COVID-19 in the subgroups of patients who had < 25%, 25–50%, and > 50% lung involvement (Table S3), except for a decrease in frequency of admission to the intensive care unit in the group of patients with 25–50% lung involvement (0.0% vs. 10.9%; $p=0.024$).

The administration of the probiotic did not exert a significant effect on the course of COVID-19 in subgroups of patients delineated based on age younger or older than 65 years (Table S4). In addition, no significant differences were observed between patients with serum CRP levels less

Table 2 Change in the values of the main biomarkers between the beginning (point 1) and end (point 2) of the trial in patients who received probiotics (PRO group) and those who did not (CON group)

Group	PRO group ($n=99$)			CON group ($n=101$)			p^{**}	p^{**}
Biomarker	1	2	p^{*}	1	2	p^{*}	1	2
Lung lesion volume, %	50 [50–75]	50 [50–75]	0.453	50 [50–75]	50 [50–75]	0.547	0.393	0.570
C-reactive protein, mg/L	66 [24–116]	3 [1–5]	< 0.001	58 [28–108]	3 [1–5]	< 0.001	0.784	0.680
White blood cells, 10^9/L	5.5 [4.0–7.7]	8.2 [6.7–10.0]	< 0.001	6.1 [4.6–9.7]	8.6 [6.3–11.9]	< 0.001	0.035	0.210
Neutrophils, 10^9/L	4.1 [2.6–5.9]	6.2 [4.2–7.4]	0.002	4.6 [2.9–8.5]	6.3 [4.5–9.5]	0.019	0.053	0.231
Lymphocytes, 10^9/L	1.0 [0.7–1.4]	1.4 [1.0–1.9]	< 0.001	1.0 [0.7–1.3]	1.2 [0.9–1.8]	< 0.001	0.740	0.656
Platelets, 10^9/L	226 [171–272]	311 [250–392]	< 0.001	236 [168–316]	316 [227–398]	0.001	0.697	0.615
ESR, mm/L	25 [21–28]	20 [14–25]	0.001	24 [20–27]	20 [14–24]	0.001	0.446	0.903
Creatinine, µmol/L	96 [80–110]	83 [76–94]	< 0.001	96 [84–109]	83 [75–100]	< 0.001	0.632	0.752
ALT, U/L	30 [20–42]	40 [32–64]	< 0.001	31 [22–46]	37 [22–82]	< 0.001	0.367	0.516
AST, U/L	35 [29–49]	36 [25–51]	0.626	35 [28–48]	43 [22–53]	0.404	0.548	0.530
Albumin, g/L	41 [39–44]	37 [31–43]	0.450	41 [40–44]	35 [30–37]	0.221	0.230	0.270
Total bilirubin, µmol/L	9 [6–11]	8 [6–10]	0.605	10 [7–12]	11 [9–14]	0.579	0.386	0.051
LDH, U/L	541 [453–687]	453 [392–564]	< 0.001	533 [410–698]	441 [384–568]	< 0.001	0.578	0.538
Ferritin, µg/L	442 [224–639]	469 [347–793]	0.617	436 [208–749]	501 [211–779]	0.067	0.923	0.593
Fibrinogen, g/L	6.0 [5.1–7.4]	3.5 [2.8–3.9]	< 0.001	5.8 [4.7–7.4]	3.5 [2.9–4.5]	< 0.001	0.283	0.376
Potassium, mmol/L	4.5 [4.1–4.9]	5.1 [4.5–5.5]	0.037	4.4 [4.1–4.7]	4.9 [4.6–5.4]	0.034	0.433	0.849

ESR erythrocyte sedimentation rate, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *LDH* lactate dehydrogenase

*Difference between the beginning (point 1) and end (point 2) of the trial within the groups

**Difference between groups at the beginning (point 1) and end (point 2) of the trial

Table 3 Maximum or minimum values of the main biomarkers during the trial

	PRO group ($n=99$)	CON group ($n=101$)	p
The maximum value during the trial			
Lung lesion volume, %	50 [50–75]	50 [50–75]	0.531
C-reactive protein, mg/L	83 [53–125]	74 [45–128]	0.398
White blood cells, 10^9/L	8.9 [7.5–12.0]	10.0 [7.3–14.2]	0.268
Neutrophils, 10^9/L	7.0 [5.3–10.0]	7.6 [5.0–11.6]	0.500
ESR, mm/L	28 [24–32]	27 [22–30]	0.132
Creatinine, µmol/L	96 [83–110]	99 [84–112]	0.509
ALT, U/L	38 [23–85]	43 [33–74]	0.964
AST, U/L	43 [30–59]	44 [29–58]	0.396
Total bilirubin, mmol/l	10 [7–12]	11 [9–14]	0.313
LDH, U/L	652 [542–793]	633 [464–830]	0.394
Ferritin, µg/L	489 [321–762]	518 [212–988]	0.753
Fibrinogen, g/L	6.3 [5.4–7.6]	6.3 [4.9–7.7]	0.504
The minimum value during the trial			
Albumin, g/L	37 [31–42]	34 [30–37]	0.057
Lymphocytes, 10^9/L	0.7 [0.6–1.1]	0.9 [0.5–1.2]	0.189

ESR erythrocyte sedimentation rate, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *LDH* lactate dehydrogenase

or greater than 60 mg/L on admission (Table S5) (this is the cut-off value for severe systemic inflammation according to the local guidelines [26]). However, a more pronounced decrease in lymphocyte count was observed in the PRO group among patients with CRP level less than 60 mg/L.

There were no cases of infections caused by these probiotic strains.

Diarrhea in COVID-19

Diarrhea on admission was observed in 23 (11.5%) patients; no significant difference was observed between the PRO and CON groups in this regard (Table 1). It was observed that diarrhea ceased earlier in patients who received the probiotic than in those who did not (Table 1).

Hospital-acquired diarrhea developed in 14 (7.0%) patients, and only in those who received antibiotics (14/154 (9.1%) vs. 0/46 (0.0%); $p=0.034$). Probiotic intake in the general cohort tended to prevent the development of hospital-acquired diarrhea. However, a significant difference was observed in the group that received only one antibiotic [$n=71$; hospital-acquired diarrhea incidence: 0 vs. 4 (12.5%); $p=0.023$]. The probiotic was not found to be effective in preventing hospital-acquired diarrhea in patients who received more than one antibiotic simultaneously or sequentially ($n=83$; hospital-acquired diarrhea incidence: 4 (10.5%) vs. 6 (13.3%); $p=0.696$).

Intake of the probiotic did not significantly affect the duration of hospital-acquired diarrhea (Table 1).

Liver Injury in COVID-19

Signs of liver injury were observed in 95 (47.5%) patients on admission, with no significant difference between the PRO

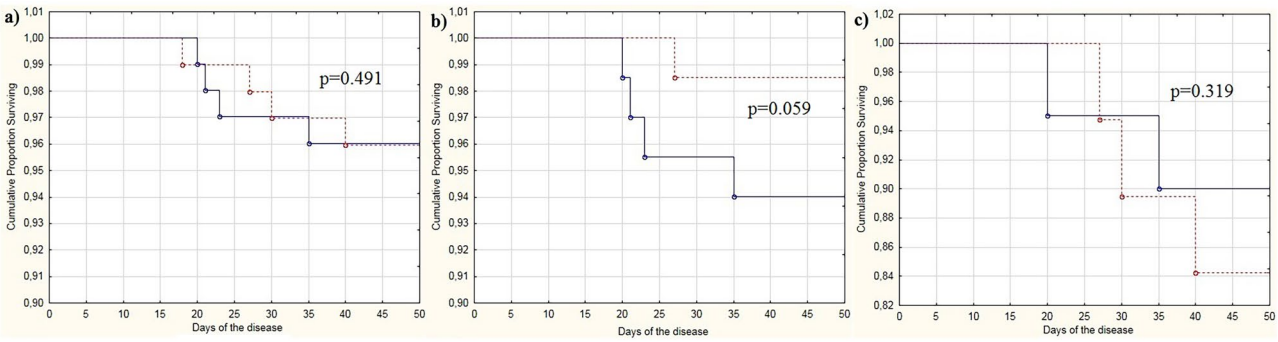

Fig. 2 Survival curves for patients with coronavirus disease (COVID-19) who received the probiotic (dotted line) and those who did not (control group) (solid line): **(a)** all patients, **(b)** patients with cardiovascular diseases, and **(c)** patients with diabetes mellitus

Table 4 Patients with abnormal liver biomarker values

	Group PRO ($n=99$)	Group CON ($n=101$)	p
Patients with abnormal liver biomarkers on admission			
ALT > ULN (45 U/L) on admission, *n* (%)	21 (21.2%)	26 (25.7%)	0.450
ALT > 3ULN on admission, *n* (%)	2 (2.0%)	1 (1.0%)	0.549
AST > ULN (45U/L) on admission, *n* (%)	27 (27.3%)	27 (26.7%)	0.932
AST > 3ULN on admission, *n* (%)	3 (3.0%)	1 (1.0%)	0.303
Total bilirubin > ULN (21 µmol/L) on admission, *n* (%)	4 (4.0%)	4 (4.0%)	0.977
Serum albumin < ULN (35 g/L) on admission, *n* (%)	10 (10.1%)	4 (4.0%)	0.889
ALP > ULN (360 U/L) on admission, *n* (%)	3 (3.0%)	3 (3.0%)	0.980
GGT > ULN (60 U/L) on admission, *n* (%)	11 (11.1%)	15 (14.9%)	0.432
Any abnormal liver biomarker, *n* (%)	45 (45.5%)	50 (49.5%)	0.566
Patients with progression of pre-admission liver injury			
Increase in the ALT level during the trial in those with high ALT levels at admission, *n* (%)	12 (12.1%)	12 (11.9%)	0.958
Increase in the AST level during the trial in those with high AST levels at admission, *n* (%)	4 (4.0%)	10 (9.9%)	0.105
Any progression of pre-admission liver injury, *n* (%)	15 (15.2%)	16 (15.8%)	0.893
Patients who developed liver injury after admission			
ALT > ULN during the trial, *n* (%)	25 (25.3%)	15 (14.9%)	0.066
ALT > 3ULN during the trial, *n* (%)	5 (5.1%)	11 (10.9%)	0.128
AST > ULN during the trial, *n* (%)	18 (18.2%)	9 (8.9%)	0.055
AST > 3ULN during the trial, *n* (%)	1 (1.0%)	4 (4.0%)	0.182
Total bilirubin > ULN during the trial, *n* (%)	0 (0.0%)	2 (2.0%)	0.159
Serum albumin < ULN during the trial, *n* (%)	5 (5.1%)	6 (5.9%)	0.783
ALP > ULN during the trial, *n* (%)	0 (0.0%)	2 (2.0%)	0.159
GGT > ULN during the trial, *n* (%)	3 (3.0%)	5 (5.0%)	0.488
Any liver injury after admission, *n* (%)	32 (32.3%)	23 (22.8%)	0.130

ALT alanine aminotransferase, *ULN* upper limit of normal, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transferase

and CON groups. Liver injury progressed during hospitalization in 31 (15.5%) of these patients, and developed after admission in 55 (27.5%) other patients; probiotic intake did not prevent liver injury in any of these cases (Table 4).

Discussion

The aim of our study was to evaluate the effect of a multi-strain probiotic on mortality, disease course, respiratory function, diarrhea, and liver injury in COVID-19 patients in a randomized controlled trial. We could not confirm the findings of an Italian retrospective study which reported that probiotic supplementation could reduce mortality in a cohort of COVID-19 patients [23]. It should be noted that the probiotic composition we used was different from that used by the Italian research group, though it is unlikely that this difference in composition can explain the difference in study outcomes. Mortality was significantly higher in the above mentioned study than in the present study (22% vs. 4%). This significant difference in mortality between the two studies may be explained by the different strategies used for COVID-19 management. The Italian researchers mainly administered hydroxychloroquine, lopinavir, azithromycin, and tocilizumab in accordance with the clinical recommendations of that time (the study was performed between March and April 2020). In the present study, lopinavir was not used, azithromycin and hydroxychloroquine were used less frequently, and almost all patients were administered with dexamethasone and anticoagulants.

The second factor responsible for the difference in mortality may be the differences in ethnicity, resulting in differences in the interaction between the virus and the host, as well as the differences in the strain composition in different countries. A third factor may be selection bias that is characteristic of retrospective studies. In addition, the severity of disease could have differed among patients in the two studies.

The differences in mortality between the patients who received and did not receive probiotics almost reached the limits of significance in the subgroup of patients with

chronic cardiovascular diseases (1.5% vs. 6.0%; $p = 0.059$). Notably, there were no patients with chronic cardiovascular diseases in the above mentioned study by Ceccarelli et al. [23]. It is interesting that gut microbiota has been shown to play a role in the development of heart failure [27]. A study with a larger sample size should be conducted to examine the effect of probiotics on mortality in this subgroup of patients and to test the hypothesis that probiotics may reduce mortality in patients with chronic cardiovascular diseases.

The probiotic did not have a significant effect on the course of the disease, inflammatory biomarkers, and renal dysfunction in our study. Moreover, this finding was observed not only in the general cohort of patients, but also in the following subgroups: subgroups of patients with chronic cardiovascular diseases, diabetes, and different volumes of lung damage; subgroups delineated based on age younger or older than 65 years; and subgroups defined based on the presence or absence of severe systemic inflammation on admission.

In our study, the administration of probiotics shortened the duration of COVID-19-associated diarrhea. This result is in agreement with the results of a retrospective study by d'Ettorre et al. [24]. It should be noted that the combination of probiotics used in the two studies differed. The causes of diarrhea in COVID-19 patients were heterogeneous [6]; viral diarrhea which develops in the early days of the disease as well as antibiotic-associated diarrhea which develops later and often in the hospital (hospital-acquired diarrhea) were observed. We also studied the effect of the multi-strain probiotic on the incidence and duration of hospital-acquired diarrhea. Hospital-acquired diarrhea developed only in those patients who received antibiotics, which may confirm its antibiotic-associated nature. Although hospital-acquired diarrhea was less common among patients who received the probiotic than among those who did not, this difference was not significant in the general cohort of patients. However, it was significant in the subgroup of patients who received only one antibiotic. Additionally, the administration of the probiotic did not affect the duration of hospital-acquired diarrhea.

Thus, in our study, probiotics for COVID-19 showed small but distinctly positive effects; these effects included a shortening of the duration of viral diarrhea by an average of 2 days, and the prevention of hospital-acquired diarrhea in patients who received a single antibiotic.

The bacteria included in the probiotic therapy used in this study have been reported to be useful in the treatment of antibiotic-associated diarrhea [28] and acute viral diarrhea [29, 30] in previous trials. These effects may be associated with their ability to form biofilms on the surface of the gut epithelium, which prevents colonization by *Clostridioides difficile* [31]. This in turn inhibits the growth and toxinogenesis of these and other pathogenic bacteria [32]. Intestinal viral infections cause increased permeability of the intestinal barrier [33]; probiotic bacteria have been reported to reduce the permeability of the intestinal barrier, and normalize the functioning of ion transporters in the epithelial cell membrane [34]. Thus, our results in patients with COVID-19 are consistent with the findings from the studies cited above.

The main strength of our study is that this is the first randomized controlled study investigating the effect of probiotics on a wide range of indicators in COVID-19. In addition, we analyzed these effects not only in the general cohort of patients, but also in subgroups within the cohort. We also performed a detailed analysis of the effects of probiotic supplementation on liver injury associated with COVID-19. However, there are several limitations to our study. This was a single-center open-label study, and did not include a placebo group. We used a probiotic that was different from those used in earlier studies, which may also be interpreted as a limitation. In addition, it was not possible to differentiate between the adverse effects of the probiotic and COVID-19-related signs due to the pronounced polymorphism of the manifestations of this disease. However, there were no cases of infections caused by these probiotic strains in our study.

New randomized controlled trials that include populations from different countries are needed to confirm our findings.

Conclusion

In conclusion, the studied probiotic did not have a significant effect on mortality and changes in most biomarkers in COVID-19 patients. However, the probiotic showed potential as treatment for diarrhea associated with COVID-19, and for the prevention of diarrhea in patients who receive a single antibiotic as part of COVID-19 treatment. A larger study on the effect of probiotics in COVID-19 patients with chronic cardiovascular diseases should be conducted to test the hypothesis that probiotics may reduce mortality in this cohort of patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12602-021-09858-5.

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Author Contribution The idea and design of the study were developed by Vladimir Ivashkin and Elena Poluektova. Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by Roman Maslennikov, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of Data and Material The datasets analyzed during the current study may be available from the corresponding author on reasonable request.

Declarations

Ethics Approval The study was approved by the local ethics committee (Conclusion №. 34-20 of September 9, 2020) in accordance with the Declaration of Helsinki.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Patients' personal data are not published.

Conflicts of Interest The authors declare no competing interests.

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