ORIGINAL ARTICLE

Intestinal microbiota composition of children with infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and multisystem inflammatory syndrome (MIS-C)

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Abstract

Microbiota composition may play a role in the development, prognosis, or post-infection of COVID-19. There are studies evaluating the microbiota composition at the time of diagnosis and during the course of COVID-19, especially in adults, while studies in children are limited and no study available in children with multisystem inflammatory syndrome in children (MIS-C). This study was planned to compare intestinal microbiota composition in children diagnosed with MIS-C and acute COVID-19 infection with healthy children. In this prospective multicenter study, 25 children diagnosed with MIS-C, 20 with COVID-19 infection, and 19 healthy children were included. Intestinal microbiota composition was evaluated by 16 s rRNA gene sequencing. We observed changes of diversity, richness, and composition of intestinal microbiota in MIS-C cases compared to COVID-19 cases and in the healthy controls. The Shannon index was higher in the MIS-C group than the healthy controls ($p < 0.01$). At phylum level, in the MIS-C group, a significantly higher relative abundance of Bacteroidetes and lower abundance of Firmicutes was found compared to the control group. Intestinal microbiota composition changed in MIS-C cases compared to COVID-19 and healthy controls, and *Faecalibacterium prausnitzii* decreased; *Bacteroides uniformis*, *Bacteroides plebeius*, *Clostridium ramosum*, *Eubacterium dolichum*, *Eggerthella lenta*, *Bacillus thermoamylovorans*, *Prevotella tannerae*, and *Bacteroides coprophilus* were dominant in children with MIS-C. At species level, we observed decreased *Faecalibacterium prausnitzii*, *and* increased *Eubacterium dolichum*, *Eggerthella lenta*, and *Bacillus thermoamylovorans in children* with MIS-C and increased *Bifidobacterium adolescentis* and *Dorea formicigenerasus* in the COVID-19 group. Our study is the first to evaluate the microbiota composition in MIS-C cases. There is a substantial change in the composition of the gut microbiota: (1) reduction of *F. prausnitzii* in children with MIS-C and COVID-19; (2) an increase of *Eggerthella lenta* which is related with autoimmunity; and (3) the predominance of *E. dolichum* is associated with metabolic dysfunctions and obesity in children with MIS-C.

Conclusions: Alterations of the intestinal microbiota might be part of pathogenesis of predisposing factor for MIS-C. It would be beneficial to conduct more extensive studies on the cause-effect relationship of these changes in microbiota composition and their effects on long-term prognosis.

What is Known:
• *Microbiota composition may play a role in the development, prognosis, or post-infection of COVID-19.*
• *However, the number of studies on children is limited, and no study on multisystem inflammatory syndrome in children is currently available (MIS-C).*
What is New:
• *In individuals with MIS-C, the composition of the gut microbiota changed dramatically.*
• *Decreased Faecalibacterium prausnitzii have been observed, increased Eggerthella lenta, which was previously linked to autoimmunity, and predominance of Eubacterium dolichum which was linked to metabolic dysfunction and obesity.*

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Abbreviations

ARDS	Acute respiratory distress syndrome
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
COVID-19	Coronavirus disease 2019
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IL-6	Interleukin 6
MIS-C	Multisystem inflammatory syndrome in children
LEFSe	Linear discriminant analysis effect size
rt-PCR	*Reverse transcription–polymerase chain reaction*
STORMS	Strengthening the Organization and Reporting of Microbiome Studies

Introduction

By the end of February 2022, the COVID-19 pandemic had maintained its severity despite diverse strategies and increased vaccination rates worldwide [1]. Since the beginning of the pandemic, children and adolescents have accounted for a relatively small proportion of total COVID-19 cases compared with adults [2]. Disease burden and the age distribution of SARS-CoV-2 infection among children and adolescents has varied according to the variants in circulation, prevention strategies applied to combat the pandemic, and the vaccination rates in a community [2, 3]. Hospitalization, intensive care unit stays, and mortality rates have also been lowered in children, and the majority of patients have been shown to have an underlying illness [4, 5]. However, COVID-19 can cause long-term complications, such as multiple systemic inflammatory syndrome in children (MIS-C) and long-COVID [6–9]. MIS-C is a severe disease condition which characterized by fever, rash, conjunctivitis, gastrointestinal symptoms, and shock due to myocardial dysfunction in children who have SARS-COV-2 positivity or a history of exposure approximately 4–6 weeks before the onset of symptoms [2, 10]. Although genetic and immune system-related risk factors are thought to play a role in the pathogenesis of MIS-C, they have not been fully elucidated and the underlying etiology appears to be multifactorial. An autoimmune-mediated inflammatory process after infection, a cytokine storm initiated by a superantigen response, and a dysregulated immune response to exposure to SARS-CoV-2 viral antigens have been proposed as theories to explain its pathogenesis [11, 12]. Immunological mechanisms, such as humoral and cellular adaptive immunity and innate response,

play roles in the development of systemic inflammatory syndrome [11]. Recent studies have also suggested that specific HLA haplotypes could be more frequently associated with the presence of this disease [13].

It has been shown that infections, especially of the gastrointestinal and respiratory systems, have effects on microbiota composition [14]. It is thought that microbiota composition may play a role in the development, prognosis, or post-infection of COVID-19 [15]. It has also been suggested that microbial interactions and competition, especially in the nasopharynx of children colonized by more viruses and bacteria compared with adults, may reduce the infectious potential of SARS-CoV-2 [16]. Some studies have evaluated microbiota composition at the time of diagnosis and during the course of COVID-19, especially in adults; however, few studies on microbiota composition in children have been conducted [17–19]. Moreover, no previous study has investigated microbiota composition in MIS-C. Therefore, this study aimed to compare intestinal microbiota composition in children diagnosed with MIS-C and acute COVID-19 infection with healthy children.

Materials and methods

This prospective multicenter (i.e., five centers in four cities) study aimed to evaluate the microbiota composition in patients diagnosed with MIS-C or SARS-CoV-2, and a control sample of healthy children aged between 3 and 14 years. This clinical study was planned and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, patient rights regulations, and ethical committees. Permission for the study was obtained from the Clinical Research Ethics Committee of Eskisehir Osmangazi University's Faculty of Medicine based on Decision Number 12 on March 2, 2021. Permission for the clinical study was also obtained from the Ministry of Health in Turkey. Written informed consent was obtained from all parents and children prior to their inclusion in the study. The study was supported by Eskisehir Osmangazi University Scientific Research Project (TTU-2021–1750). The results of the study and flow chart are shown according to Strengthening the Organization and Reporting of Microbiome Studies (STORMS) [20] (Fig. 1).

COVID-19 was diagnosed according to SARS-CoV-2 rt-PCR positivity in nasopharyngeal swab samples. To diagnose MIS-C, we used the Centers for Disease Control and Prevention (CDC) guidelines [21]. The exclusion criteria for the patient and control groups were as follows: the presence of chronic disease or routine medications; use of antibiotics/probiotics in the last 8 weeks; and treatment related to MIS-C or COVID-19 before the study samples were collected. The age, gender, height, body weight, and
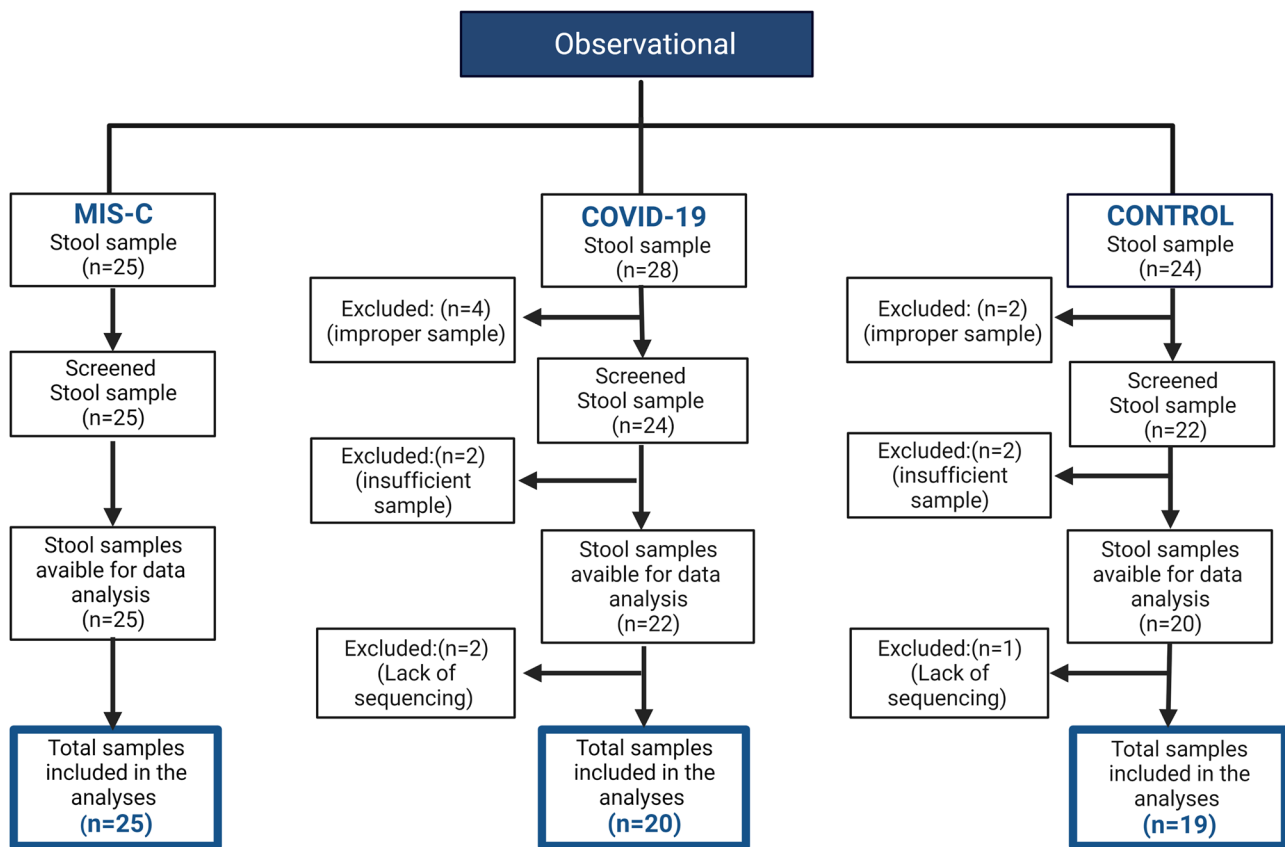
Fig. 1 Flow chart of the study groups

body mass index (BMI) of all cases at the time of admission were recorded, and anthropometric parameters were based on percentile scales according to the age group. Obesity was defined as a BMI at or above the 95th percentile and overweight at or above the 85th percentile on the CDC's sex-specific age-for-age BMI growth charts [22]. Symptoms and signs were noted in all cases based on a detailed physical examination. SARS-CoV-2 rt-PCR and serological tests (SARS-CoV-2 IgM and/or IgG) were noted. At the time of diagnosis, white blood cell count, absolute neutrophil count, absolute lymphocyte count, platelet count, absolute eosinophil count, blood biochemistry (i.e., urea nitrogen, creatinine, transaminases, albumin, cardiac markers, and lactate dehydrogenase), inflammation biomarkers (i.e., serum procalcitonin, erythrocyte sedimentation rate, fibrinogen, ferritin, C-reactive protein, and IL-6 levels), and coagulation parameters (aPTT, PT, INR, and D-dimer) were extracted from medical records. We enrolled 24 aged-matched healthy children (matched with 25 children with MIS-C) served as the control group (Fig. 1). IgM/IgG rapid diagnosis kits (KIT-19 SARS-CoV-2 IgG/IgM Rapid Test Cassette, Devaju®, Turkey) were used to evaluate whether the healthy children to be

included in the study had the disease previously; only children with negative test results were enrolled.

Stool samples were obtained from the participants at the time of their enrolment before initiation of any COVID-19/MIS-C-related therapy. The stool samples of at least 5 ml were taken in 50 cc Falcon tubes and then immediately frozen. The Falcon tubes were stored upright at − 80 °C without any treatment. All samples were delivered to the laboratory where the DNA analysis was conducted in accordance with cold chain requirements every 3 months.

Fecal DNA extraction, sequencing, and bioinformatics analysis QuickGene (Kurabo, Japan) was used to extract DNA from the stool samples. First, 25 mg of each stool sample was transferred to a homogenization tube with 250 μl of tissue lysis (MDT) solution. To homogenize the solution, 15 mg of 0.1 mmø glass beads or 10 1.0 mmø zirconia beads were added to the tube and then homogenized for 2×120 s at 5,000 rpm. After the sample was homogenized, 25 μl of Proteinase K (EDT) solution was added and incubated at 56 °C for 60 min. The tube was then centrifuged at 15,000 g for 10 min at room temperature. After centrifugation, 200 μl of supernatant was transferred to a 1.5 ml microtube. Then

180 μl of cell lysis (LDT) solution was added and vortexed for 15 s. The microtube was left to incubate at 70 °C for 10 min. In the next step, 240 μl of 99% cold ethanol was added and vortexed for 15 s. The entire contents of the microtube were transferred to a QuickGene (Kurabo, Japan) filtered cassette, where washes and elutions were performed following the instrument protocol. Three washes were performed using 750 μl of wash buffer (WDT) solution. Based on the results of the extraction process, bacterial 16S ribosomal RNA (rRNA) gene target sequencing was performed from the materials obtained in the study. The resulting genomic DNA was amplified using 16S V3-V4 314F-860R primer sets, and library preparation was performed using a Nextera XT DNA library preparation kit and indices (Illumina, CA, USA). The amplicon library was cleaned by selecting large fragments (AMPure XP, Beckman Coulter). It was then normalized and aggregated. After the library was prepared, the NovoSeq 6000 (Illumina, CA, USA) instrument was used to run the sequencing.

Pair-ended Illumina reads (2×250) were transferred to the QIIME2 environment [23]. All samples had a sequence depth greater than $100 \times$, and no samples were omitted from the run. Quality clipping, chimera detection, and read cleaning were implemented using the QIIME2 Dada2 pipeline (via q2-dada2) [24]. Amplicon sequence variants (ASVs) generated by Dada2 were mapped to the GreenGenes (http://greengenes.lbl.gov) database [25, 26]. The phyloseq object was created from qiime2 artifact files in the R 4.1 environment [27, 28]. Alpha diversity metrics (Chao-1 diversity and Simpson index) were calculated from the phyloseq object using the microbiome R package. Significant differences between groups were calculated using the Kruskal–Wallis rank sum test. Beta diversity was computed by phyloseq, including the Bray–Curtis, Jaccard, unweighted UniFrac, and weighted UniFrac distance metrics. Beta diversity statistical significance between groups was calculated using a PERMANOVA test via the Adonis function in the vegan R package. Intergroup p values were calculated using the Kruskal–Wallis test. Specific differences between groups were determined by differential abundance analysis using the Deseq2 R package [29]. Linear discriminant analysis effect size (LEFSe) analyses were performed between groups to determine statistically significant taxonomies [30].

Statistical analysis

The SPSS Statistical Package for the Social Sciences (Illinois, CA, USA) program was used for the statistical analysis. Descriptive statistical methods (i.e., median in percentage) Pearson's chi-square, Kruskal–Wallis, and ANOVA tests were conducted to compare the qualitative data; $p < 0.05$ was accepted as statistically significant correlations between

data. In this study, the R program was used to evaluate the microbiota analysis.

Results

A total of 64 children (27 boys, 37 girls) were included in the study (20 COVID-19 cases [10 boys, 10 girls], 25 MIS-C cases [16 boys, 9 girls], and 19 healthy children [11 boys, 8 girls]). There was no statistical difference between the study groups in terms of gender ($p > 0.05$). The median age was 7 years in the MIS-C group, 9.5 years in the COVID-19 group, and 8 years in the control group (Table 1). The age distribution of the MIS-C, COVID-19, and control groups was statistically similar ($p < 0.05$). In the MIS-C group, five children were obese (> 95th percentile), and nine children were overweight (85–95th percentile). It was observed that in the COVID-19 group, five subjects were obese (> 95th percentile) and seven subjects were overweight (85–95th percentile).

Diagnoses were based on SARS-CoV-2 rt-PCR positivity in 95% of the COVID-19 cases and antigen positivity in one case. In the MIS-C cases, 24% ($n = 6$) were SARS-CoV-2 rt-PCR positive, 52% ($n = 13$) were SARS-CoV-2 IgG positive, 28% ($n = 7$) were both SARS-CoV-2 IgG and SARS-CoV-2 IgM positive, and antigen positivity was detected in one case. There was a history of contact in 10 cases (40%) in the MIS-C group.

In the COVID-19 cases, fever was the most common symptom (75%, $n = 15$), followed by cough (50%, $n = 10$). Other symptoms were diarrhea (40%, $n = 5$), tachypnea (40%, $n = 5$), runny nose (40%, $n = 5$), myalgia (35%, $n = 7$), headache (35%, $n = 7$), and abdominal pain (30%, $n = 6$). In the COVID-19 group, positive results were found in 13 cases (65%) of chest radiography and in 7 cases (35%) of computerized thorax tomography.

Fever was present in all MIS-C cases. The most common symptoms in the MIS-C patients were abdominal pain (72%, $n = 18$), diarrhea (56%, $n = 14$), and myalgia (48%, $n = 12$), followed by headache (36%, $n = 9$), tachypnea (20%, $n = 5$), cough (16%, $n = 4$), and runny nose (8%, $n = 2$). Most cases had more than one symptom. The most common sign was rash (44%). Other pathological findings were symptoms of conjunctivitis (24%), respiratory (20%), and neurological (8%) symptoms. Most patients in the study population had multisystem involvement. The most common system involvements were hematologic (72%) and gastrointestinal (64%). Other system involvements were cardiovascular (40%), dermatological (32%), respiratory (20%), and neurological (8%) (Table 1). The most common laboratory parameters in the MIS-C cases were elevated serum C-reactive protein levels in 16 cases (64%), elevated serum fibrinogen levels in 13 cases (52%),

Table 1 Demographic and clinical findings of the study groups

	MIS-C (*n* = 25)	COVID-19 (*n* = 20)	Control (*n* = 19)
Age/years	7 (5–10)	9.5 (5.2–11)	8 (7–10)
Body weight (kg)	28 (20–43)	41.5 (20.5–59.7)	32 (26–37)
Height (cm)	124 (115–145)	137 (114–156)	133 (120–140)
Body mass index (BMI) (kg/m^2)	17.8 (15.9–22.0)	20.2 (15.7–24.1)	18.9 (16.6–21.9)
Presence of overweight/obesity (*n*/%)			
Overweight (85–95th p)	9/25 (36)	7/20 (3)	0/19 (0)
Obese (> 95th p)	5/25 (20)	5/20 (25)	0/19 (0)
Symptoms* (*n*/100)			
Fever	25/25 (100)	15/20 (75)	
Cough	4/25 (16)	10/20 (50)	
Runny nose	2/25 (8)	5/20 (40)	
Diarrhea	14/25 (56)	5/20 (40)	
Tachypnea	5/25 (20)	5/20 (40)	
Myalgia	12/25 (48)	7/20 (35)	
Abdominal pain	18/25 (72)	6/20 (30)	
Headache	9/25 (36)	7/20 (35)	
System involvement** (*n*/%)			
Hematological	18/25 (72)		
Gastrointestinal	16/25 (64)		
Cardiovascular	10/25 (40)		
Dermatological	8/25 (32)		
Respiratory	5/25 (20)		
Neurological	2/25 (8)		

*Most patients had more than one symptom. **Most patients had more than one system involvement

and elevated D-dimer levels in 12 cases (48%). Other markers were lymphopenia in 9 cases (36%), high ferritin in 8 cases (32%), high BNP in 8 cases (32%), high erythrocyte sedimentation rate in 4 cases (16%), high INR in 4 cases (16%), elevated serum IL-6 levels in 4 cases (16%), high levels of procalcitonin in 3 cases (12%), high levels of LDH in 1 case (4%), high levels of neutrophils in 2 cases (8%), and elevated liver function in two cases (8%).

Intestinal microbiota analysis

Alpha and beta diversity Although the median observed taxonomic units (OTUs) value observed in the stool samples taken from the MIS-C group was lower than those in the control and SARS-CoV-2 groups, no statistical difference was found in OTU levels, the Chao-1 index, and Simpson index ($p > 0.05$). Species dominance distribution between the 25th and 75th percentile range in both stool sample data was found to be greater in MIS-C cases compared to other groups (Fig. 2a). The Shannon index is a measure of richness and uniformity that considers the entropy used in the study. It was higher in the MIS-C cases compared with the control group ($p < 0.01$) (Fig. 2b). A statistical difference in the stool samples was found between the groups in the results of the Bray–Curtis (Fig. 2c), Jaccard (Fig. 2d), weighted UniFrac (Fig. 2e), and unweighted UniFrac (Fig. 2f) baseline coordinate analysis (PCoA) ($p = 0.001$).

The MIS-C group showed the following phylum levels: *Firmicutes* (46%), *Bacteroidetes* (32%), *Proteobacteria* (11%), *Actinobacteria* (7%), and *Verrucomicrobia* (3%). In the control group, they were as follows: *Firmicutes* (64%), *Bacteroidetes* (23%), *Actinobacteria* (6%), and *Proteobacteria* (5%). In the SARS-CoV-2 group, the levels were as follows: *Firmicutes* (51%), *Bacteroidetes* (25%), *Proteobacteria* (12%), *Actinobacteria* (9%), and *Verrucomicrobia* (2%). Regarding the presence of *Bacteroidetes*, no difference was observed between the SARS-CoV-2 group and the control group; however, *Bacteroidetes* were higher in the MIS-C group compared with the other two groups.

Microbiota composition is shown according to genera and species levels in Fig. 3. At genera levels, the dominant genus in the healthy controls was *Faecalibacterium* (16%), which was decreased in the MIS-C (9%) group and the SARS-CoV-2 (8%) group. *Bacteroides*, *Eggerthella*, and *Clostridium* genera were significantly higher in the MIS-C group (21%, 10%, and 21%, respectively) than in the SARS-CoV-2 group (16%, 6%, and 2%, respectively) and the control group (15%, 2%, and 15%, respectively) (Fig. 3a).
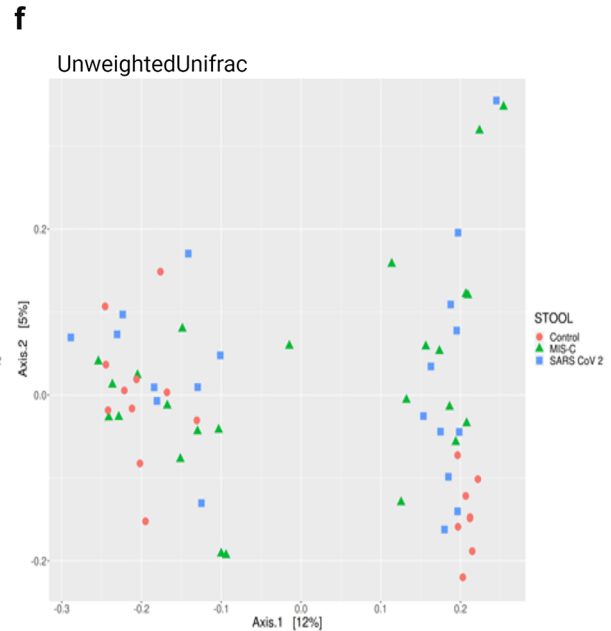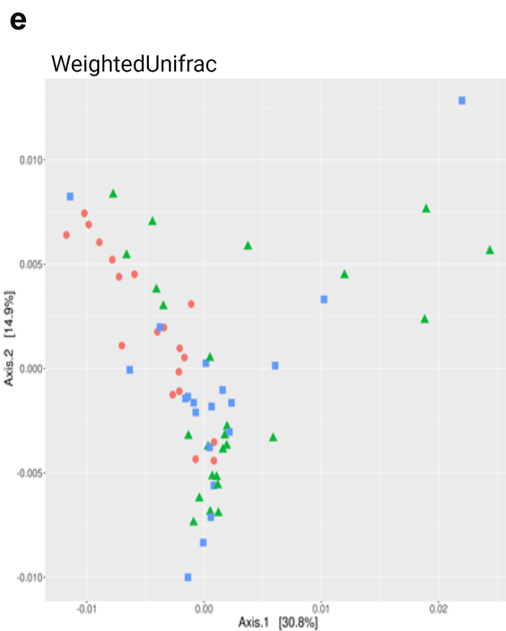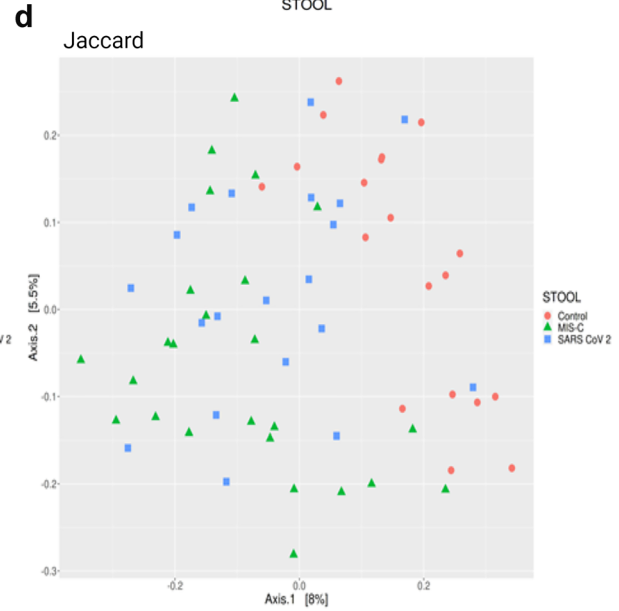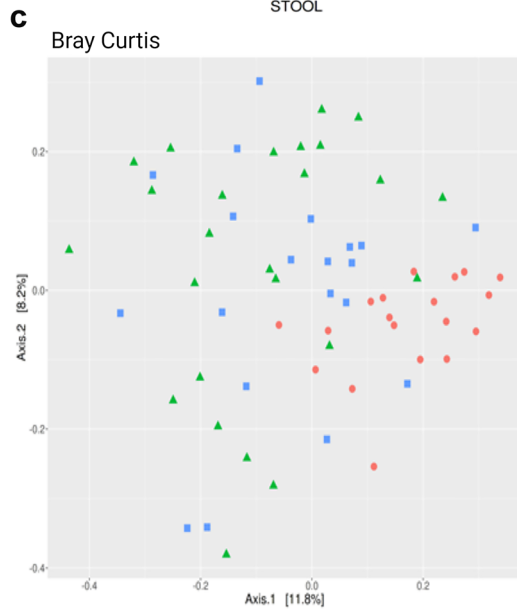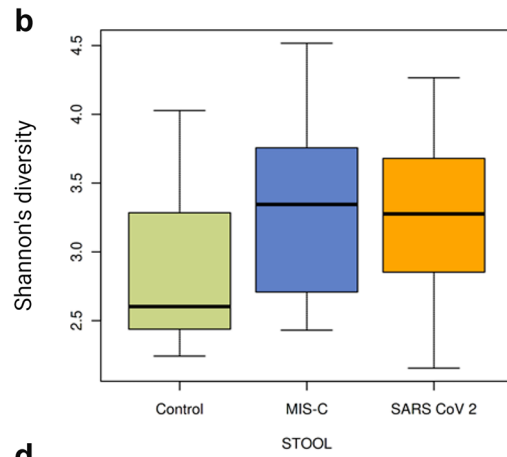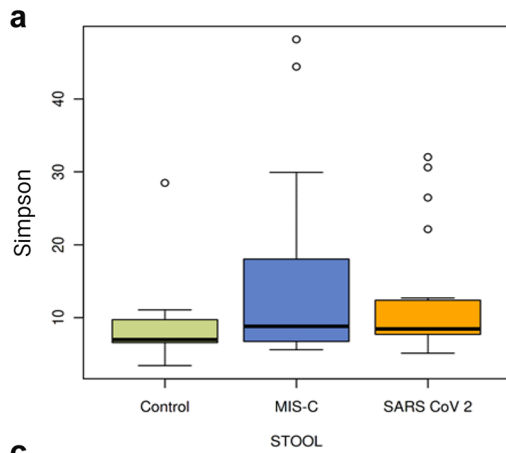
◄**Fig. 2** Alpha and beta diversity plots to visualize the difference in microbiota structure between the MIS-C, SARS-CoV-2, and control groups. Simpson index comparison between the study groups. There was no significant difference in stool sample data between the groups in the study ($p > 0.05$). **a** Shannon index comparison between the study groups. Shannon index was higher in stool sample data of MIS-C cases when the control group ($p < 0.01$). **b** PCoA 2D plots of beta diversity analysis of the MIS-C, SARS-CoV-2, and control groups. Each dot represents a stool sample. Red circle, green triangle, and blue square represent control, MIS-C, and SARS-CoV-2 cases, respectively. **c**–**f** Between-sample dissimilarities were measured by Bray–Curtis distances (**c**), Jaccard distance (**d**), weighted UniFrac distances (**e**), and unweighted UniFrac distances (**f**). A statistical difference in the stool samples was found between the groups in the results of the Bray–Curtis (**c**), Jaccard (**d**), weighted UniFrac (**e**), and unweighted UniFrac (**f**) baseline coordinate analysis (PCoA) ($p = 0.001$)

At the species level, we observed abundances of *Faecalibacterium prausnitzii*, *Gemmiger formicilis*, *Ruminococcus bromii*, *Bifidobacterium adolescentis*, *Lactobacillus ruminis*, *Butyricicoccus pullicaerocum*, *Collinsella aerofaciens*, *Ruminococcus callidledikans*, and *Dorea formicians* in the healthy controls compared with the MIS-C group. The most abundant species in the MIS-C group were *Bacteroides uniformis*, *Bacteroides plebeius*, *Clostridium ramosum*, *Eubacterium dolichum*, *Eggerthella lenta*, *Bacillus thermoamylovorans*, *Prevotella tannerae*, and *Bacteroides coprophilus*. The SARS-CoV-2 group showed an increase in the relative abundance of *Bacteroides coprophilus*, *Bifidobacterium adolescentis*, *Dorea formicigenerans*, *Ruminococcus albus*, and *Clostridium piliforme* (Fig. 3b).

In this study, a LEfSe analysis (LDA threshold value > 2, $p < 0.05$) was used to determine significant bacterial compositions between groups. The results of the LEfSe analysis in the MIS-C group and the control group are shown in Fig. 4a. At the genus level, in the MIS-C group, *Bacteroides*, *Eggerthella*, and *Prevotella* were dominant, whereas *Faecalibacterium*, *Gemmiger*, *Ruminococcus*, *Butyricicoccus*, *Collinsella*, and *Dorea* were found to be dominant in the control group. At the species level, *Bacteroides uniformis*, *Bacteroides plebeius*, *Clostridium ramosum*, *Eubacterium dolichum*, *Eggerthella lenta*, *Bacillus thermoaamylovorans*, *Prevotella tannerae*, and *Bacteroides coprophilus* were most abundant in the MIS-C group. In the control group, *Faecalibacterium prausnitzii*, *Gemmiger formicilis*, *Bifidobacterium adolescentis*, *Ruminococcus bromii*, *Lactobacillus ruminis*, *Butyricicoccus pullicaerocum*, *Collinsella aerofaciens*, *Ruminococcus callidus*, and *Dorea formicigenerans* were found to be dominant (Fig. 4a).

The results of the LEfSe analysis conducted to compare the SARS-CoV-2 group and the control group are shown in Fig. 4b. At the genus level, *Lachnospiraceae_Clostridium* was dominant in the SARS-CoV-2 group. *Faecalibacterium* and *Ruminococcus* were dominant in the control group. At the species level, in the SARS-CoV-2 group, *Bacteroides*

coprophilus, *Eubacterium dolichum*, *Bacteroides uniformis*, and *Clostridium piliforme* were dominant. *Faecalibacterium prausnitzii*, *Ruminococcus callidus*, and *Lactobacillus ruminis* were dominant in the control group (Fig. 4b).

The results of the LEfSe analysis of three groups (MIS-C, SARS-CoV-2, and the controls) were also compared and *Bacteroides uniformis*, *Bacillus thermoaamylovorans*, and *Eubacterium dolichum* were the most abundant species in the MIS-C group at the species level. *Bacteroides coprophilus*, *Bifidobacterium adolescentis*, *Dorea formicigenerans*, *Ruminococcus albus*, and *Clostridium piliforme* were the most abundant species in the SARS-COV-2 group. In the control group, *Faecalibacterium prausnitzii*, *Gemmiger formicilis*, *Ruminococcus bromii*, *Lactobacillus ruminis*, *Butyricicoccus pullicaerocum*, and *Ruminococcus callidus* were dominant (Fig. 4c).

Discussion

In our study, we first showed that the diversity, richness, and composition of intestinal microbiota in the MIS-C group at the time of diagnosis differed from those in the healthy controls. These findings indicate that the composition of microbiota can deteriorate during the course of infection. Previous studies have evaluated microbiota composition at the time of diagnosis of COVID-19, during the course of the disease, and at post-disease follow-up, especially in adults. Chen et al. [31] showed that microbiota richness did not return to normal levels 6 months after recovery in adult patients with COVID-19; however, another study showed that dysbiosis improved rapidly after the disease, and there was no difference from the healthy control group [17]. Geographical and demographic differences have been observed in previous studies on the recovery of microbiota composition after COVID-19 [32]. Our study is the first to be conducted in children with MIS-C, and the fact that both alpha and beta diversity indicators and their relative abundance were different in the MIS-C cases compared with the healthy controls supported that microbiota composition changed in the MIS-C group.

In our study, the relative abundance of Bacteroidetes increased in the MIS-C group, and the Firmicutes:Bacteroides ratio decreased significantly in the MIS-C group compared with the healthy control group and the SARS-CoV-2 group. Endotoxins and metabolites of microorganisms causing inflammation in the lung have been shown to enter the bloodstream, causing an increase in the relative abundance of Bacteroidetes and a decrease in the relative abundance of Firmicutes in the intestinal composition [33]. In addition, previous findings showed that *Firmicutes* decreased and *Bacteriodes* increased in Kawasaki disease [34]. We also found
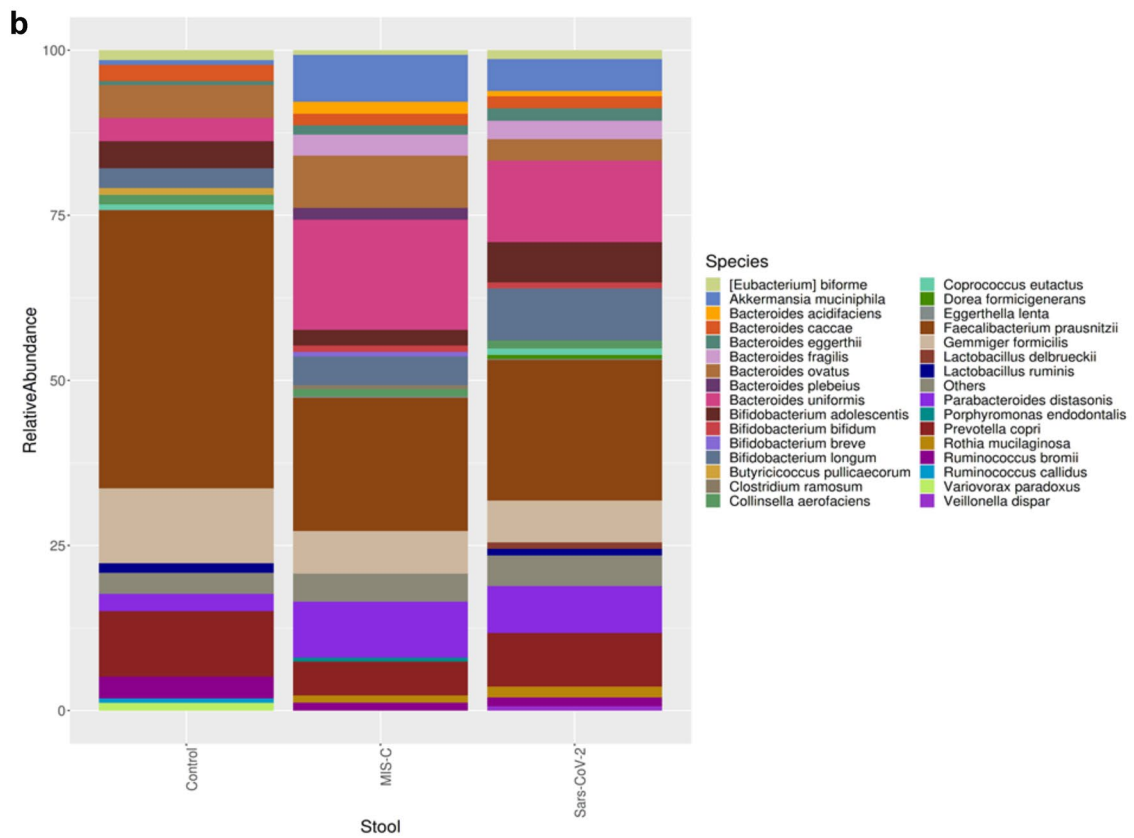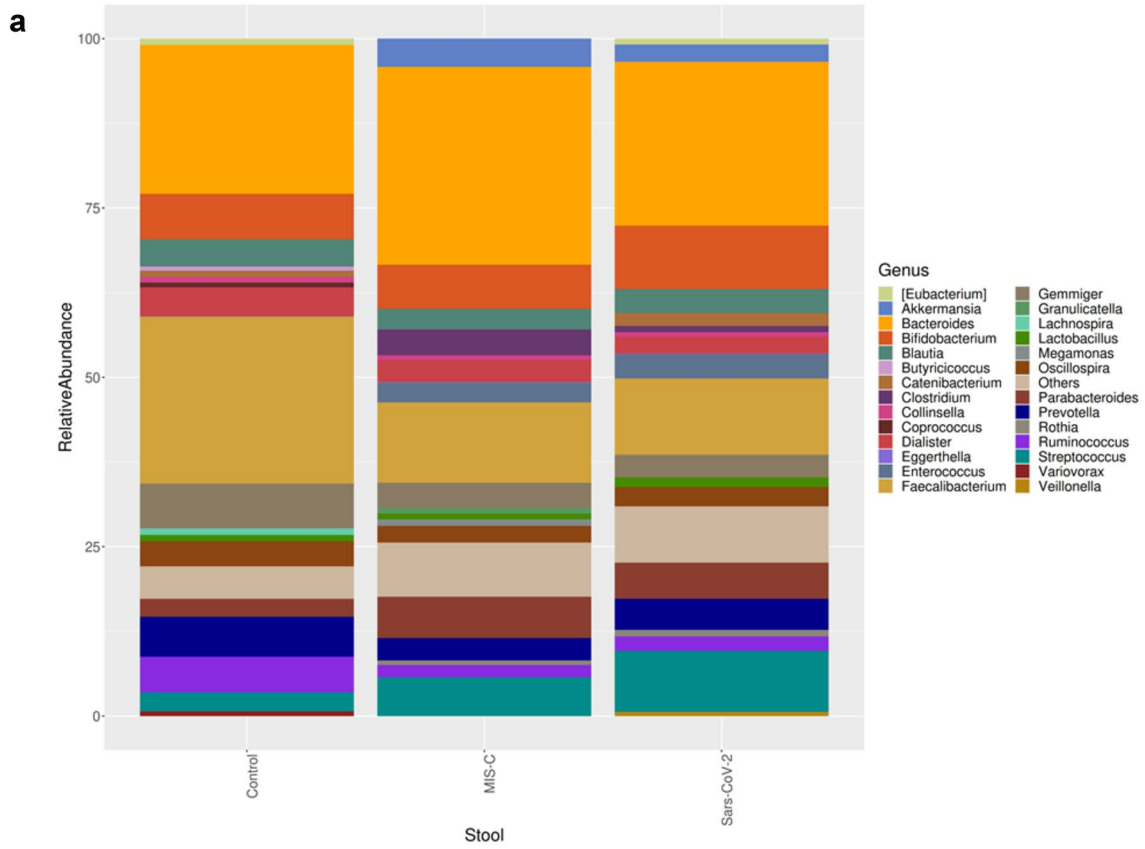
◀**Fig. 3** Intestinal microbiota composition of the study groups at species and genus levels. Bacterial community relative abundance analysis at the genus (**a**) and species (**b**) levels (relative abundance > 1%; bacteria with relative abundances < 1% were pooled in the "others" category and sorted by total concentration)

an increased percentage of *Proteobacteria* (11%) in children with MIS-C compared to healthy controls (5%).

Compared with the healthy children in the MIS-C group, the dominant genera were *Bacteroides*, *Eggerthella*, and *Prevotella*, and the dominant species were *Bacteroides uniformis*, *Bacteroides plebeius*, *Clostridium ramosum*, *Eubacterium dolichum*, *Eggerthella lenta*, *Bacillus thermoamylovorans*, *Prevotella tannerae*, and *Bacillus thermoamylovorans*. The findings also showed that *Faecalibacterium prausnitzii* decreased in the MIS-C group compared with the healthy control group. *F. prausnitzii*, which is a notable member of intestinal microbiota, has positive effects on the immune system, as well as anti-inflammatory properties. Previous studies have shown that decreased *F. prausnitzii* could be considered a microbiological marker of gastrointestinal tract inflammation [35]. Decreased *F. prausnitzii* has been reported in cystic fibrosis [36], inflammatory bowel disease [37–39], hypertension [40], multiple sclerosis [41], obesity [42], type 2 diabetes [43, 44], rheumatoid arthritis [45], celiac disease [46], and in adults with COVID-19 [18, 47, 48]. In our study, we also found that *F. prausnitzii* decreased in the intestinal microbiota composition of the cases in the MIS-C group, which could be accepted as an indicator of gastrointestinal system involvement/inflammation in MIS-C cases.

Our findings also showed an increase at the genus level in the microbiota element *Prevotella* in the MIS-C group. *Prevotella* strains are anaerobic gram-negative bacteria of the *Bacteroidetes* phylum, and they are among the natural elements of microbiota composition. A previous study found a relationship between *Prevotella* strains and periodontitis, bacterial vaginitis, rheumatoid arthritis, and systemic and metabolic disorders [49]. In a study conducted on obese children in Mexico, the findings showed that *Prevotella* strains were increased [50]. It was concluded that there might be a relationship between *Prevotella* dominance and high zonulin levels in obese patients with colorectal cancer [51]. Although zonulin levels were not included in the present study, they were found to be high in MIS-C cases in a previous study [52]. In rheumatoid arthritis patients, it was shown that *Prevotella* activated the TLR-2 receptor of intestinal epithelial cells, stimulated the release of proinflammatory cytokines, such as IL-1β, IL-6, and IL-23, supported the activation of Th17 cells that lead to IL-17 production, and played a role in inflammation [53, 54]. In our study, we found that *Prevotella* at the genus level and *Prevotella*

tannerae were significantly dominant in the intestine at the species level. Considering inflammation in MIS-C disease, further studies are needed on the genus and species of *Prevotella* in MIS-C cases.

Eggerthella lenta is an anaerobic, non-spore-forming gram-positive bacillus that is among the natural members of the gut microbiota [55]. Previous studies have shown that Th17 activation may have effects on related inflammatory diseases, familial Mediterranean fever, some infections, and autoimmune diseases [56–61]. Crohn's disease, ulcerative colitis, multiple sclerosis, and rheumatoid arthritis were found to be higher in the Behçet's disease group than in the *Eggerthella* control group at the genus level [62, 63]. In our study, a significant increase was observed in the MIS-C group at both the genus (*Eggerthella*) and species levels (*Eggerthella lenta*). Considering autoimmunity and the Th17 pathway in the pathogenesis of MIS-C [64], our findings suggest that an increase in *Eggerthella lenta* may be associated with the pathogenesis. We also found that *Eubacterium dolichum* was dominant in both the MIS-C and COVID-19 groups in our study. The *E. dolichum* strain is predominant in obesity and metabolic dysfunctions, which have been defined as risk factors for MIS-C [65–68]. Similarly, the predominance of *Clostridium ramosum*, which was previously shown to increase in metabolic syndrome, obesity, and COVID-19 cases, in our study, it was thought to support the relationship between obesity and metabolic disorders and MIS-C [59, 69–73].

The clinical picture of MIS-C may be characterized by an exaggerated immune response and cytokine storm in children and adolescents, who may be genetically susceptible after exposure to SARS-CoV-2 [74]. Previous findings showed that genetic predisposition, environmental factors, and intestinal microbiota play roles in the development of abnormal immune responses and inflammation in many diseases [75–77]. It has also been shown that microbiota composition is impaired in many diseases (i.e., dysbiosis), but research has yet to determine whether these disorders were causes or effects. The findings of our study did not provide evidence for a cause–effect relationship between existing microbiota in MIS-C cases.

Previous studies on adults have reported a reduction in microbial diversity and a possible association between increased intestinal dysbiosis and disease severity in COVID-19-positive patients compared with healthy controls [78]. One hypothesis regarding the less severe course of COVID-19 infection in children compared with adults is that their oropharyngeal, nasopharyngeal, lung, and/or gastrointestinal microbiota are different from those of adults [16]. It has been suggested that, especially in the nasopharynx of children colonized with more viruses and bacteria compared with adults, microbial interactions and competition may reduce the infection potential of SARS-CoV-2 [16, 79].
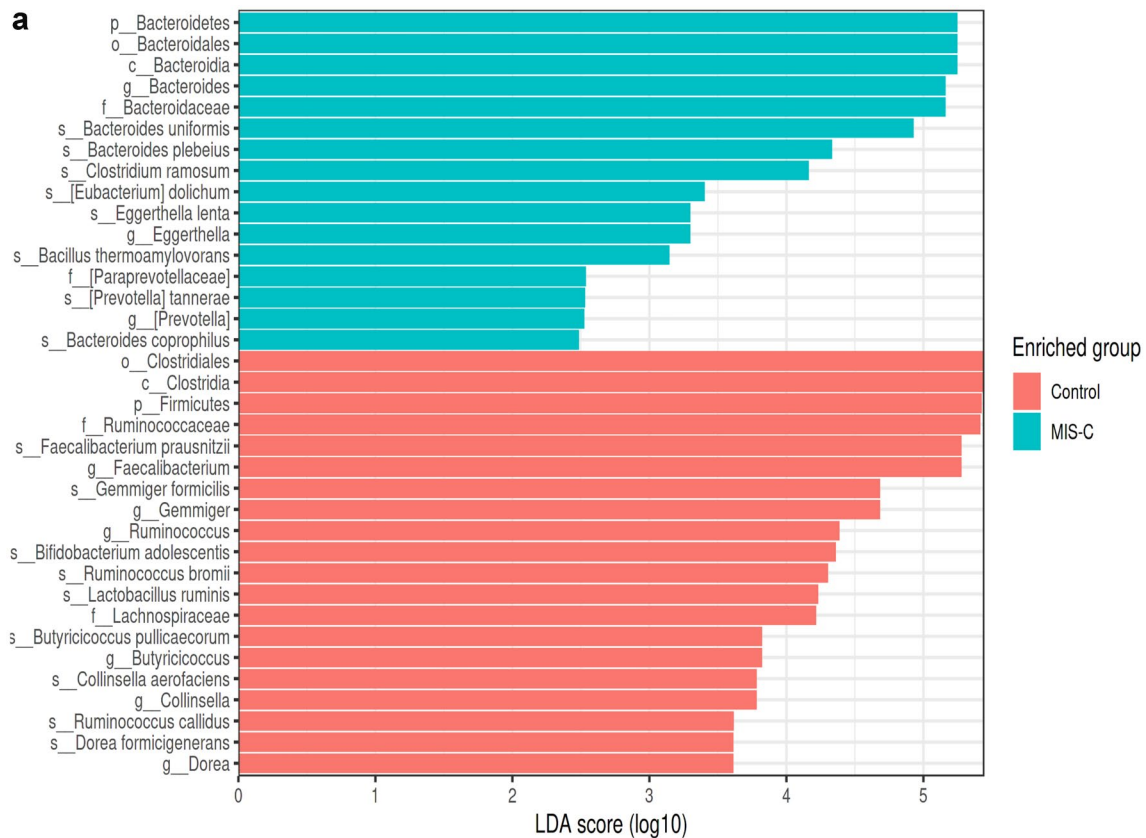
Fig. 4 LEfSe analysis graphics showing bacterial taxa that were significantly different in abundance between the study groups. **a–c** LefSe analysis was performed to identify differentially abundant taxa for which the LDA scores are shown. Only species and functional modules with LDA effect size > 2 and FDR-corrected p value < 0.05 were plotted. Horizontal bars represent log.10 converted LDA score indicated by vertical dotted lines. Different colors represent different groups. p—phylum, c—class, o—order, f—family, g—genus, s—species. LEfSe analysis in stool samples for the MIS-C and control groups (control group (red), MIS-C (green)). **a** At the genus level, in the MIS-C group, *Bacteroides* (LDA score = 5.162, p = 0.018), *Eggerthella* (LDA score = 3.297, p = 0.017), and *Prevotella* (LDA score = 2.514, p = 0.038) were dominant, whereas *Faecalibacterium* (LDA score = 5.265, p = 0.0008), *Gemmiger* (LDA score = 4.666, p = 0.012), *Ruminococcus* (LDA score = 4.368, p = 0.000), *Butyricicoccus* (LDA score = 3.815, p = 0.002), *Collinsella* (LDA score = 3.752, p = 0.045), and *Dorea* (LDA score = 3.608, p = 0.045) were found to be dominant in the control group. At the species level, *Bacteroides uniformis* (LDA score = 4.926, p = 0.023), *Bacteroides plebeius* (LDA score = 4.332, p = 0.046), *Clostridium ramosum* (LDA score = 4.160, p = 0.046), *Eubacterium dolichum* (LDA score = 3.401, p = 0.023), *Eggerthella lenta* (LDA score = 3.296, p = 0.032), *Bacillus thermoaamylovorans* (LDA score = 3.146, p = 0.023), *Prevotella tannerae* (LDA score = 2.523, p = 0.038), and *Bacteroides coprophilus* (LDA score = 2.460, p = 0.041) are most abundant in the MIS-C group. In the control group, *Faecalibacterium prausnitzii* (LDA score = 5.265, p = 0.000), *Gemmiger formicilis* (LDA score = 4.666, p = 0.012), *Bifidobacterium adolescentis* (LDA score = 4.344, p = 0.008), *Ruminococcus bromii* (LDA score = 4.286, p = 0.000), *Lactobacillus ruminis* (LDA score = 4.231, p = 0.046), *Butyricicoccus pullicaerocum* (LDA score = 3.815, p = 0.002), *Collinsella aerofaciens* (LDA score = 3.752, p = 0.045), *Ruminococcus callidus* (LDA score = 3.609, p = 0.008),

and *Dorea formicigenerans* (LDA score = 3.608, p = 0.000) were found to be dominant (**a**). LEfSe analysis in stool samples for the SARS-CoV-2 and control groups (control group (red), SARS-CoV-2 (blue)). **b** At the genus level, *Lachnospiraceae_Clostridium* (LDA score = 2.648, p = 0.039) was dominant in SARS-CoV-2 cases, while *Faecalibacterium* (LDA score = 5.168, p = 0.016) and *Ruminococcus* (LDA score = 4.316, p = 0.037) were dominant in the control group. At the species level, in the SARS-CoV-2 group, *Bacteroides coprophilus* (LDA score = 3.629, p = 0.002), *Eubacterium dolichum* (LDA score = 3.261, p = 0.012), *Bacteroides uniformis* (LDA score = 4.834, p = 0.011), and *Clostridium piliforme* (LDA score = 2.642, p = 0.039) were dominant, while *Faecalibacterium prausnitzii* (LDA score = 5.168, p = 0.016), *Ruminococcus callidus* (LDA score = 3.632, p = 0.003), and *Lactobacillus ruminis* (LDA score = 4.184, p = 0.013) were dominant in the control group (**b**). LEfSe analysis in stool samples for the MIS-C, SARS-CoV-2, and control groups (control group (red), MIS-C group (green), SARS-CoV-2 (blue)). **c** *Bacteroides uniformis* (LDA score = 4.820, p = 0.024), *Bacillus thermoaamylovorans* (LDA score = 3.040, p = 0.048), and *Eubacterium dolichum* (LDA score = 3.297, p = 0.002) were most abundant species in children with MIS-C at species level. *Bacteroides coprophilus* (LDA score = 3.331, p = 0.008), *Bifidobacterium adolescentis* (LDA score = 4.408, p = 0.016), *Dorea formicigenerans* (LDA score = 3.560, p = 0.000), *Ruminococcus albus* (LDA score = 3.049, p = 0.017), and *Clostridium piliforme* (LDA score = 2.450, p = 0.0130) were most abundant species in children with SARS-COV-2. In the control group, *Faecalibacterium prausnitzii* (LDA score = 5.201, p = 0.000), *Gemmiger formicilis* (LDA score = 4.524, p = 0.019), *Ruminococcus bromii* (LDA score = 4.169, p = 0.000), *Lactobacillus ruminis* (LDA score = 4.231, p = 0.046), *Butyricicoccus pullicaerocum* (LDA score = 3.660, p = 0.002), and *Ruminococcus callidus* (LDA score = 3.499, p = 0.000) were found to be dominant (**c**)
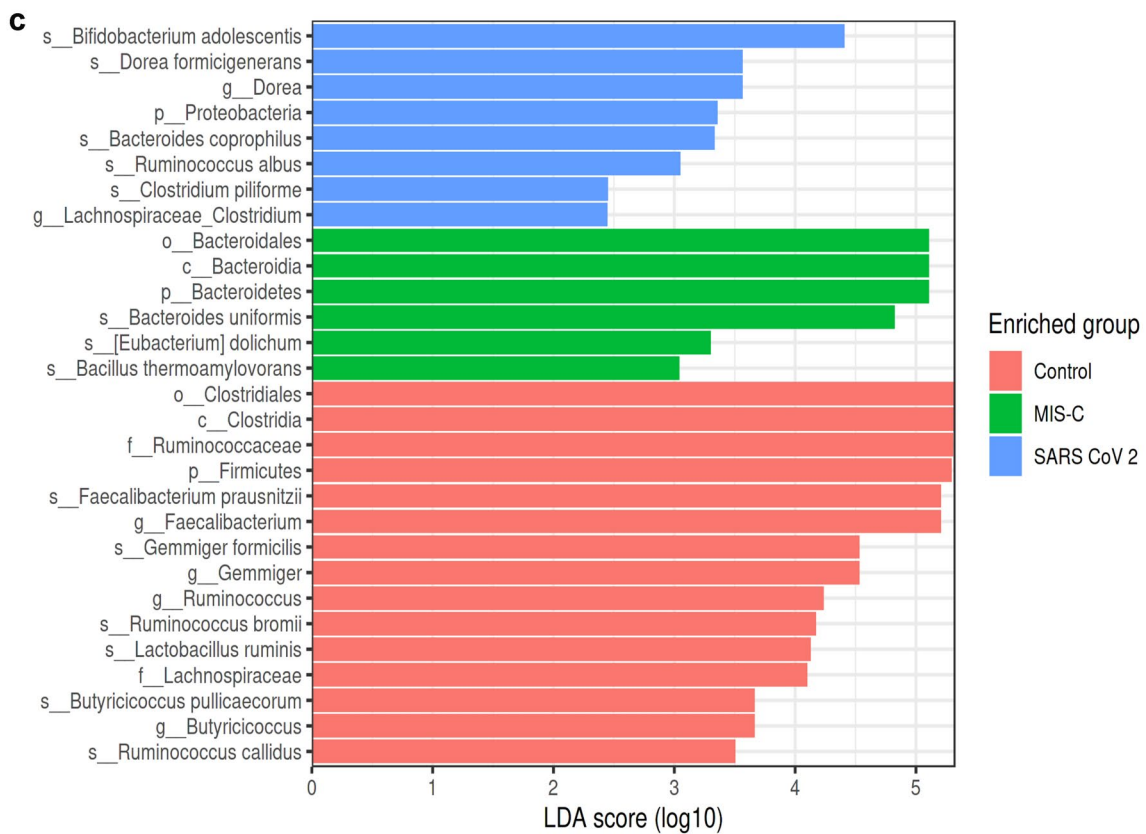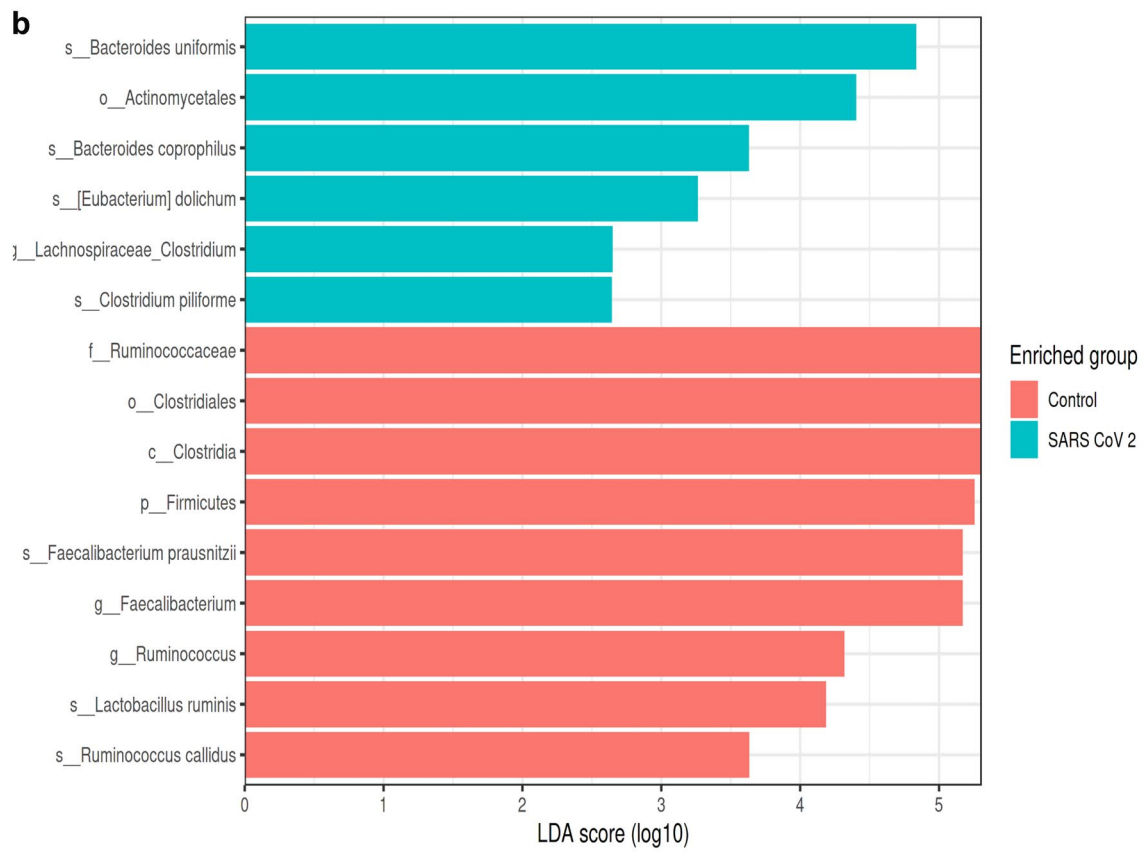
Fig. 4 (continued)

In our study, different strains were determined by intestinal microbiota analysis at the time of diagnosis of COVID-19 in the children in that group, which had not been reported in other studies. In the COVID-19 group, the taxa *Eubacterium*, *Faecalibacterium prausnitzii*, *Roseburia*, and *Lachnospiraceae*, which are common in healthy children, were detected less frequently. The comparison of the COVID-19 group with the MIS-C group showed further differences. Previous studies have shown that *Faecalibacterium* species and bacterial diversity in general are reduced in patients infected with SARS-CoV-2 [72, 73, 78, 80]. A negative correlation was found between the presence/increase of *Faecalibacterium prausnitzii*, which has anti-inflammatory properties and is one of the important features of healthy gut microbiota, and disease severity [72, 81]. It has also been shown that microbiota change depending on inflammation in COVID-19 cases [18, 72, 82]. In another study, butyrate-producing *F. prausnitzii*, *Clostridum butyricum*, *C. leptum*, and *Eubacterium rectale* were significantly reduced during COVID-19 infection [81]. A reduction in short-chain fatty acids may downregulate ACE-2, one of the key receptors of the SARS-CoV-2 virus [83]. Experimental studies have shown that the depletion of ACE-2 promoted intestinal epithelial damage and increased sensitivity to inflammation [84]. In COVID-19 cases, microbiota may play a role in the pathogenesis of acute lung injury by various mechanisms, including the direct translocation of bacteria from the gut to the lung and the immune-modulating effects of microbe-related metabolites [85]. Increased levels of *Enterobacteriaceae* and *Lachnospiraceae* were observed in patients with severe ARDS [86]. These results suggest that microbiota can be used as a potential marker in the development and follow-up of ARDS in COVID-19 cases [87]. Previous research has shown that children with respiratory tract infections have changes in their gut microbiome [88]. Hasegawa et al. [89] discovered that infants with the Enterobacter/Veillonella-dominant profile had the lowest proportion of bronchiolitis and those with the Bacteroides-dominant profile had the highest. De Maio and colleagues recently conducted a comparative analysis in hospitalized infants younger than 6 months with acute bronchiolitis caused by RSV or non-RSV, and found no statistically significant differences in intestinal microbiota composition in children with bronchiolitis caused by RSV or non-RSV [90].

The long-term effects of SARS-CoV-2 infection in children are little understood [91]. To date, the majority of investigations have focused on adult populations. However, a subset of children appear to recover from their initial COVID-19 infection but have signs and symptoms and may be diagnosed with long-COVID [92]. Severe acute infection might be related with chronic symptoms; however, symptoms might be also related with chronic inflammation, autoimmunity, chronic endotheliopathy, microthrombosis, or viral persistence [91]. Microbiota composition changes might also play a role in long-COVID. In our study, we did not enroll children with long-COVID and potential further studies will clarify this interaction.

Our study has the following limitations. We collect a single fecal sample at the diagnosis, and it is not possible to evaluate the cause of consequence of. Our research was conducted during the period when the Alpha variant and the first part of the Delta variant were apparent in SARS-CoV-2 infection. Moreover, our results did not include the period when the Delta and Omicron variants were predominant. Because many factors affect microbiota composition in children, it was not possible to ensure that all the children in our study groups were homogeneously similar. Geography, diet, culture, traditions, physiological variations, and changes in lifestyle have been shown to affect microbiota composition. Most cases included in the SARS-CoV-2 group had mild to moderate symptoms and signs, and microbiota composition or prognoses could not be evaluated in severe cases. In addition, the children in the MIS-C and SARS-CoV-2 groups and in the healthy control group have been enrolled during the restrictions applied at the global level in response to the COVID-19 epidemic (e.g., curfews, school closures, increased hand hygiene, fewer social interactions, and travel restrictions).

Conclusion

Our study is the first in the literature to show that changes occurred in intestinal microbiota composition in MIS-C cases. It is not possible to conclude whether the changes we detected in the composition of the microbiota were causes or effects. Many previous studies have shown a decreased abundance of *Faecalibacterium prausnitzii* in various intestinal diseases, similar to the children in our MIS-C group. In our study, we also found an increase in the *Eggerthella lenta* strain in the MIS-C group compared with the healthy controls. The predominance of *E. lenta* in diseases associated with abnormal Th17 activation, such as rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease, shown in previous studies supports the association of *E. lenta* with autoimmunity. In addition, it is thought that the changes detected in microbiota composition in children may affect the immune, gastrointestinal system, metabolic, and brain–intestinal axis of the child at that time, as well as in adulthood [93]. Long-term observations of these changes, which we detected for the first time in our study, and their effects on prognoses should be conducted in a larger series.

Availability of data and material Authors agree to participate all data, if requested.

Code availability Not available.

Declarations

Ethics approval Permission for the study was obtained from the Clinical Research Ethics Committee of Eskisehir Osmangazi University's Faculty of Medicine based on Decision Number 12 on March 2, 2021. Permission for the clinical study was also obtained from the Ministry of Health in Turkey.

Consent to participate Written informed consent was obtained from all parents and children prior to their inclusion in the study. This clinical study was planned and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, patient rights regulations, and ethical committees.

Consent for publication Not available.

Conflict of interest The authors declare no competing interests.

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