Response to reviewer 1

The article under review is a review article on topical issues of human microbiome research. The systematization of data on microbiome-dependent associations with various human diseases is currently important both from the fundamental and applied research. The authors consider these relationships in irritable bowel syndrome (IBS). Generally, a large amount of experimental material and meta-analyses were analyzed. The main remark, which should be supplemented by the authors, is to systematize the main experimental material presented in sections PI-IBS MICROBIOME ALTERATION and MICROBIOME-DIRECTED THERAPY in the form of tables.

The main experimental material was systematized in the form of Table 1 and Table 2.

This will significantly enhance the quality of the work carried out by the authors. Additionally, the authors should more carefully review the correspondence of all cited references in the text, especially to pay attention to references 5, 6, 84, 93, 49, and 112.

References were verified. References 5 and 6 were changed as they were not appropriate. Reference 84, 93 and 49 remained the same. There was a crossmatch between references 111 and 112 that was resolved.

Tabel 1.Alterations of the gut microbiota observed during acute gastroenteritis and during PI-IBS

Study	**Subjects/Methods**	**Sample and techniques**	**Microbiota alterations**	**Other findings**
Jalanka-Tuovinen et al,[52] 2014	11 postinfection IBS 11 postinfection bowel dysfunction 12 postinfection without bowel dysfunction 12 IBSD, 11 healthy controls Adults	16S rRNA gene phylogenetic microarray analysis with HITChip, 16S rRNA gene qPCR with group and species-specific primers of feacal sample	Index of microbial dysbiosis" comprised of 27 genus-like groups including: ↑*Bacteroidota* including various *Bacteroides and Prevotella* species ↓*Bacillota* including various uncultured *Clostridiales*, and *Clostridium* clusters	Dysbiosis was associated with bowel, not psychological symptoms Dysbiosis associated biopsy findings: ↑eotaxin, mast cells, goblet cells, ↓enterochromaffin cells Dysbiosis associated RNA expression pathways: ↑serotonin transport, condensed chromosome, B cell antigen receptor

				↓caspase
Hsiao et al,[85] 2014	7 adults with *V. cholerae* AGE history 50 healthy children 12 healthy adults	16S rRNA gene PCR, V4 region analysis of faecal sample	One week after AGE: ↑ *V. cholerae* *Streptococcus* spp *Fusobacterium* spp *Campylobacter* spp	Two months after AGE (recovery period): ↓*V. cholerae* *Streptococcus* spp *Fusobacterium* spp *Campylobacter* spp ↑species indicating recovery *Ruminococcus obeum* *Collinsella aerofasciens* *Ruminococcus torques* *Eubacterium rectale* *Faecalibacterium prausnitzii*
Ma et al,[86]	13 Adenovirus diarrhea 13 Rotavirus diarrhea 13 Astrovirus diarrhea 13 Norvirus diarrhea 6 control children	16S rRNA gene PCR, V3 region analysis of faecal sample	↓Diversity in diarrheal patients ↑*Enterococcus,* *Peptostreptococcaceae* *Incertae Sedi* *Shigella* *Weissella spp*	↓*Bacteroides vulgatus* *Bifidobacterium* *Lactobacillus* spp
Youmans et al,[87]	111 all-cause traveler's diarrhea/ 12 healthy travelers	16S rRNA gene PCR, V3 and V5 regions analysis of faecal sample	↓*Bacteroidota:Bacillota* ratio in diarrheal patients ↑Species diversity during norovirus infection ↑*Clostridium XIVb* *Bilophilia* *Alistipes* *Barnesiella,* *Roseburia* spp during norovirus infection	↑*Bacillota* phylum *Streptococcus* *Lactococcus* spp in healthy travelers (unexpected)
Patin et al,[88]	4 symptomatic and 5 asymptomatic norovirus infected adults	16S rRNA gene analysis of faecal sample	Post norovirus challenge: ↑ *Bacillota* phylum, particularly *Clostridia*	Prior to norovirus challenge: Asymptomatic patients

			↓*Bacteroidota Pseudomonadota*	had ↑*Bacteroidota* phylum and ↓*Clostridia* compared to symptomatic
Nelson et al,[89] 2012	38 norovirus infection 22 healthy controls	16S rRNA gene 454 pyrosequencing, V3-V5 regions analysis of faecal sample	A subset (approximately 1/5) patients with norovirus had: ↓diversity, ↑*Pseudomonadota* phylum *Enterobacteriaceae* family	*E. coli* diversity and virulence was not associated with norovirus infection
Cheng et al[90], 2022	COVID-19 acute and recovery phase Non COVID-19	Meta-analysis of 16S rRNA microbial data	↓*Ruminococcus Faecalibacterium Roseburia Coprococcus genus* ↑ *Fusobacterium Streptococcus in recovery/post-recovery COVID-19 compared to non-COVID 19*	↓*Clostridium clostridioforme* ↑*Bifidobacterium breve in COVID-19 compared to recovery/post-recovery COVID-19*
Liu et al,[96] 2022	68 COVID-19 patients 68 non-COVID-19 patients	Shotgun metagenomic sequencing	At 6 months follow up 76% developed Post-acute COVID-16 syndrome (PACS) -non-PACS showed recovered gut microbiome profile at 6 months comparable to that of non-COVID-19 controls ↑*Ruminococcus gnavus, Bacteroides vulgatus* and ↓ *Faecalibacterium prausnitzii* in PACS	Butyrate-producing bacteria, including *Bifidobacterium pseudocatenulatum* and *Faecalibacterium prausnitzii* showed the largest inverse correlation with PACS at 6 months

Zuo et al,[98] 2020	15 Acute COVID-patients 6 community acquired pneumonia patients 15 healthy controls	Shotgun metagenomic sequencing	-Antibiotic naïve patients ↑ *Clostridium hathewayi, Actinomyces viscosus,* and *Bacteroides nordii* compared with controls -COVID-19 with antibiotic use ↓ *Faecalibacterium prausnitzii, Lachnospiraceae bacterium 5_1_63FAA, Eubacterium rectale, Ruminococcus obeum,* and *Dorea formicigenerans* compared with COVID-19 naïve patients	Baseline abundance of *Coprobacillus, Clostridium ramosum,* and *Clostridium hathewayi* correlated with COVID-19 severity -there was an inverse correlation between abundance of *Faecalibacterium prausnitzii* and disease severity -depletion of symbionts and enrichment of opportunistic pathogens persisted after clearance of SARSCoV-2
Yeoh et al,[100] 2021	100 COVID-19 patients 78 non COVID-19 controls	shotgun sequencing total DNA extraction from stool sample	Patients with COVID-19 were depleted in *Faecalibacterium prausnitzii, Eubacterium rectale* and several bifidobacterial species, which remain low up to 30 days from disease resolution	Composition of the gut microbiota in patients with COVID-19 is concordant with disease severity and magnitude of plasma concentrations of several inflammatory cytokines, chemokines and blood markers of tissue damage
Sundin et al,[104] 2015	13 PI-IBS patients 19 general IBS patients 16 healthy controls	HITChip for mucosal and fecal microbiota	↓mucosal and faecal diversity *Bacillota* phylum including *Clostridium* clusters IV and XIVa	Reduced diversity was associated with psychological symptoms and increased

			↑*Bacteroidota* phyum including *Bacteroides* spp	activated lamina propria lymphocytes Did not find a difference in major butyrate producer abundance

Table 2. PI-IBS therapeutic options

Study	**Therapeutic intervention**	**Outcome**
Compare et al [107], 2017	*Lactobacillus casei* DG (LC-DG)+ postbiotic	↓ the inflammatory mucosal response in an ex-vivo organ culture model of PI-IBS-D
Hong et al,[108] 2019	*Lactobacillus acidophilus* LA5, *Bifidobacterium animalis* subsp. *lactis* BB12 and *Saccharomyces cerevisiae* var. *boulardii*)	↓ pro-inflammatory cytokine levels in both the control and Pi-IBS induced mice
Abbas et al,[109] 2014	*Saccharomyces boulardii*	Improved the quality of life and the cytokine profile in PI-IBS patients
Lee et al, [111] 2017	*Bifidobacterium infantis*	Restored the normal composition of gut microbiota and improved mental health among individuals with post-flood acquired IBS
Cao et al,[112] 2018	*L. rhamnosus* supernatant	Had a positive effect on SERT expression in colon tissues of rats with PI-IBS, improving IBS symptoms in PI-IBS rats
Chen et al,[113] 2022	*E. faecium* and *E. faecalis* supernatant, in PI-IBS rats.	The supernatants of B. subtilis, E. faecium, and E. faecalis can upregulate SERT expression in intestinal epithelial cells and the intestinal tissues in the rat model of PI-IBS.

Tkach et al,[115] 2022	*RCT,* low FODMAP diet + Otilonium Bromide +a multi-strain probiotic vs FMT procedure	FMT proved effectiveness in restoring normal gut microbiota and ameliorating PI-BS symptoms, compared to traditional pharmacotherapy, as well as a high degree of safety and good tolerability.
Liu et al,[116] 2021	FMT procedure	FMT can partially restore the gut dysbiosis in COVID-19 patients by increasing the relative abundance of *Actinobacteria* (15.0%) and reducing *Proteobacteria* (2.8%) at the phylum level. At the genera level, *Bifidobacterium* and *Faecalibacterium* had significantly increased after FMT.
Jin et al,[118] 2017	Rifamixin in PI-IBS rats	Rifaximin alleviated visceral hypersensitivity, recoverd intestinal barrier function and inhibited low-grade inflammation in colon and ileum of PI-IBS rats Exerts anti-inflammatory effects with only a minimal action on the overall composition and diversity of the gut microbiota
Harris et al,[119] 2019	Rifamixin vs placebo in veterans with IBS	Rifaximin was not associated with signifcant improvement in global symptoms, abdominal pain, stool frequency, urgency, bloating, or stool consistency

Tuteja et al,[120] 2019	Rifamixin vs placebo in veterans with IBS	Rifaximin was not effective in improving IBS symptoms and QOL in GW Veterans with non-constipated IBS.
Lam et al, [121] 2016	Mesalazine vs placebo	Mesalazine was no better than placebo in relieving symptoms of abdominal discomfort or disturbed bowel habit. Mesalazine did not reduce mast cell percentage area stained. A subgroup of patients with postinfectious IBS may benefit from mesalazine.
Baffuto et al,[122] 2011	Mesalazine in PI-IBS patients compared to non-infective IBS patients	Mesalazine reduced key symptoms of postinfectious irritable bowel syndrome and noninfective irritable bowel syndrome with diarrhea patients, with no statistical difference between IBS and PI-IBS
Tuteja et al,[123] 2012	Mesalazine vs placebo	There was no significant improvement in global symptoms or overall QOL with mesalazine in patients with PI-IBS.
Andresen et al,[124] 2016	Mesalazine during the AGE with Shiga-like toxin-producing *E. coli* (STEC)	Mesalazine administration during AGE with STEC might be a protective factor for PI-IBS
Dunlop et al,[125] 2003	Prednisolone vs placebo	Prednisolone does not appear to reduce the number of enterochromaffin cells or cause an improvement in symptoms in PI-IBS

Response to reviewer 2

The authors assessed the literature to review the evidence on the role of the gut microbiome in post-infectious irritable bowel syndrome. Although the manuscript was written well, I have some comments: TITLE • Please revise the title as follows: "Emerging role of gut microbiome in post-infectious irritable bowel syndrome: a literature review"

Title was changed as you recommended.

INTRODUCTION • Please state clearly the basic and clinical impressions of your study in the last paragraph of the Introduction. What problems remain unanswered? What are questions responding to? MAIN TEXT •

The aim of this review is to analyze current literature and to describe the role of the human gut microbiota on PI-IBS physiopathology. Which long-term consequences of acute enteric infections may serve as triggers to post-infectious irritable bowel syndrome and whether the acute enteric infection associated dysbiosis and its recovery can be used to predict PI-IBS development are main questions to be answered to. If there is a specific microbial siganture associated to PI-IBS- is an issue that remains under disscusion, as most studies of PI-IBS combine patients infected by varying pathogens, thus generating considerable variability of outcomes. Gut microbiota modulation and its potential therapeutic implications in PI-IBS in terms of efficacy and safety continue to be a subject of debate and highlight the need of specific treatment protocols . A better characterization of the relationship between gut-asociated dysbiosis and PI-IBS progression will lead the way to a personalized medicine and individualized management of each patient.

You should summarize your results and evidence in different tables.

The main experimental material was systematized in the form of Table 1 and Table 2.

Please cite relevant reviews, such as PMID: 32143424 and PMID: 34304786.

PMID: 32143424 was cited as reference 105

PMID: 34304786.was cited as reference 10

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	bowel dysfunction 12 postinfection without bowel dysfunction 12 IBSD, 11 healthy controls Adults	microarray analysis with HITChip, 16S rRNA gene qPCR with group and species-specific primers of feacal sample	groups including: ↑*Bacteroidota* including various *Bacteroides and Prevotella* species ↓*Bacillota* including various uncultured *Clostridiales*, and *Clostridium* clusters	psychological symptoms Dysbiosis associated biopsy findings: ↑eotaxin, mast cells, goblet cells, ↓enterochromaffin cells Dysbiosis associated RNA expression pathways: ↑serotonin transport, condensed chromosome, B cell antigen receptor ↓caspase
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