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■ RESEARCH ARTICLE

Aberrant Human Gut Microbiome Composition in COVID-19 Patients: A Meta-Analysis

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ABSTRACT: The COVID-19 pandemic has created a global emergency that calls for a better understanding of our immune defenses against SARS-CoV-2. The gut microbiome plays a vital role in regulating host immunity. Emerging evidence has suggested a possible association of the irregular gut microbiome with COVID-19. However, the heterogeneity among the results of these studies requires thorough statistical analysis. This study examines the relationship between gut microbiome composition and COVID-19 through a systematic review of published research and statistical analysis. Three databases, including PubMed, Web of Science, and Embase, were searched for articles that reported measures of gut bacterial composition in COVID-19 patients. Eleven publications containing appropriate information were selected and analyzed for alpha diversity, beta diversity, and relative abundance indexes. The results of this study suggest that COVID-19 patients are associated with abnormal microbiome composition, as reflected by a statistically significant decrease in microbial diversity compared to healthy individuals. In addition, COVID-19 patients exhibited notably decreased health-promoting bacteria. The result of this study indicates that the gut microbiome can be used as a biomarker in monitoring COVID-19 disease progress and recovery. These findings suggest restoring the immunomodulatory bacteria may serve as a promising novel adjuvant therapy for COVID-19.

KEYWORDS: Microbiology; Applied Microbiology; COVID-19; Gut Microbiome; Bacterial Diversity.

■ Introduction

COVID-19, caused by SARS-CoV-2, has rapidly spread worldwide, resulting in over 500 million infections and 6 million deaths globally since December 2019.[1] It is a respiratory illness with a spectrum of clinical implications, and the symptoms can range from mild fever and cough to severe pneumonia and multiple organ failures. Infection by SARS-CoV-2 induces an immune response to eradicate the virus. Still, plenty of evidence has suggested that an aberrant immune response is responsible for severe illness and damage to the lung and other organs. It is also common that long-term implications on the body remain after the patients have recovered from the acute phase of the disease.[2,3] The severity of this disease has elicited the development of multiple COVID-19 therapies, most of which are focused on virus clearance, such as neutralizing monoclonal antibodies and small molecule drugs targeting the viral protease or RNA polymerase. However, due to the long-lasting effects of SARS-CoV-2 infection on the human body even after viral removal, it is necessary to develop novel therapy that allows patients to recover from the severe damages caused by COVID-19 entirely. The gut microbiome is the collection of many microorganisms living in symbiosis with hosts that contribute to human health. The gut microbiome is involved in the host nutrient metabolism, drug metabolism, protection from pathogens, and maintenance of structural integrity of the intestinal mucosal barrier. Studies in recent years have shown that the gut microbiome also plays an essential role in regulating the host immune system. Maintaining a healthy gut microbiome and the imbalance of the microbiome are implicated in metabolic diseases, autoimmune and inflammatory diseases, neurodegenerative disorders, and cardiovascular illness.[4,5] The goal of this study was to systematically review the emerging evidence on the association between gut microbiome alterations and COVID-19, highlighting the potential of using gut microbiome composition as a biomarker for monitoring disease progression and treatment effectiveness and its promise as a potential new adjuvant therapy for COVID-19.

■ Methods

A systematic review of the original clinical articles was conducted to evaluate the human gut microbiome in COVID-19. The "preferred reporting items for systematic review and meta-analysis" (PRISMA) reporting guidelines were followed.[6] PubMed, Embase, and Web of Science were searched to identify articles with original data published before February 1, 2022. Search terms or keywords include: "COVID-19," "COVID-19," SARS-CoV-2 Infection," "Gastrointestinal Microbiome," and "Gut Microbiome."

Selection Criteria:

The PRISMA guidance stipulates that the literature retrieval of systematic review and meta-analysis shall comply with PICOS:

P(Population): Patients with COVID-19
I(Intervention): SARS-CoV-2 infection
C(Comparison): Healthy controls without COVID-19
O(Outcomes): Aberrant gut microbiota composition
S(Study): Observational study

Data Extraction:

Publications details, including several patients and methodological information, were extracted. The community-level measures of gut microbiota composition (using alpha and beta diversity indexes) and taxonomic findings at the phylum and

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species levels (using relative abundance indexes) were then determined. Alpha diversity provides an overview of microbial communities in individual samples. It can be compared across groups to assess the samples' richness (number of species) and uniformity (representation of each species). Beta diversity is a measure of diversity among individuals (between samples). It evaluates the similarity between the community and control samples analyzed.[7,8] For the relative abundance of microbial groups, qualitative synthesis was conducted.

Results

1235 original studies in PubMed, Embase and Web of Science databases were obtained. 11 original studies were included according to the inclusion criteria. The literature screening flowchart is shown in Figure 1.

Characteristics of Included Studies:

Of the 11 studies included, seven were from China, and the remaining four were from the United States, Italy, India, and the United Arab Emirates. A total of 436 patients with COVID-19 infection and 336 healthy people were included in the analysis. The detailed characteristics of the included studies are shown in Table 1.

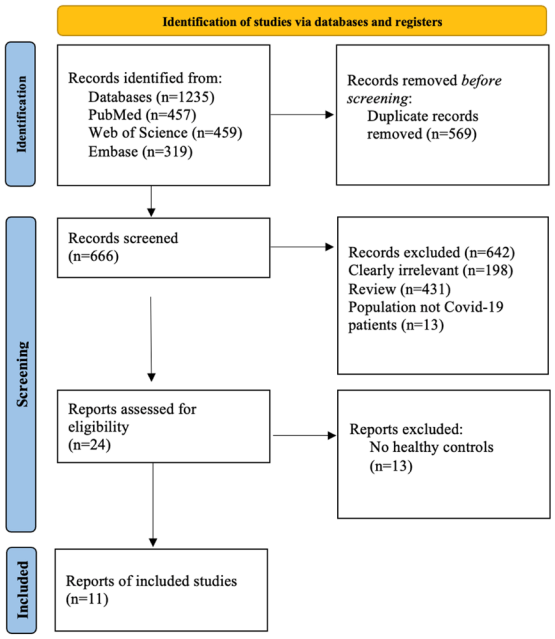

Figure 1: Screening flowchart.

Alpha Diversity:

Six of the eight studies that reported the Shannon index provided available data and were included in the analysis (230 patients and 182 controls). Although there is heterogeneity between the studies(I2=89%), the pooled estimate demonstrated a significant difference between groups (Standard Mean Difference (SMD) = −0.81; 95% *CI*, −1.56 to -0.06; p=0.03) (Figure 2). Compared with healthy people, COVID-19 patients had significantly lowered intestinal flora diversity.

Beta Diversity:

10 of the 11 studies performed the difference test based on principal component analysis. The results of the beta diversity analysis are summarized in Table 2. These studies showed that the composition of intestinal flora after infection with COVID-19 was significantly different from that of healthy people.

Relative Abundance:

All studies assessed the relative abundance of gut microbes, and 10 of 11 studies identified significant differences between patients and controls at phylum or genus levels. The differences between COVID-19 patients and healthy controls in microbial taxa spanned 14 phyla and 37 genera.

Table 1: Summary of included studies.

Study ID	Authors	Year	Country	# COVID	# Control	Alpha Diversity	Beta Diversity	Relative Abundance
1	Silan Gu et al.[9]	2020	China	30	30	Shannon Index; Chao Index	PCoA of Bray-Curtis	phylum, class, family, and genus
2	Mohammad Al Bataineh et al.[10]	2021	UAE	86	57	Shannon Index	PCoA of weighted UniFrac	genus
3	Jiabao Cao et al.[11]	2021	China	13	5	Shannon Index	PCoA of Bray-Curtis	species
4	Paolo Gaibani et al.[12]	2021	Italy	69	69	Simpson Index	PCoA of Bray-Curtis	family, genus and species
5	Sabine Hazan et al.[13]	2021	USA	50	20	Shannon Index; Simpson Index	weighted UniFrac	family, and genus
6	Mahejibin Khan et al.[14]	2021	India	30	10	Simpson Index; ACE Index; Fisher index	-	phylum, family, genus, and species
7	Sijia Li et al.[15]	2021	China	47	19	-	PCoA of Bray-Curtis	phylum, family, genus, and species
8	Zhigang Ren et al.[16]	2021	China	24	48	Shannon Index	-	phylum, and genus
9	Yun Kit Yeoh et al.[17]	2021	China	87	78	Shannon Index	-	phylum, and species
10	Tuoyu Zhou et al.[18]	2021	China	13	13	Shannon Index; Chao Index	-	phylum, family, genus and species
11	Zhonghan Sun et al.[19]	2022	China	63	8	Shannon Index	PCoA of unweighted Unifrac	phylum, and species

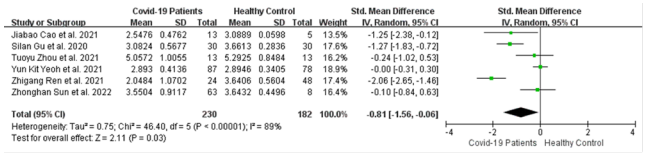

Figure 2: Compared with healthy controls, forest plots of Alpha Diversity richness estimators in the gut microbiota of patients with COVID-19.

Because the microbial taxa in the human intestine are very complex, and the microbial taxa studied in the 11 included articles are quite different, I chose to compare the typical flora in the top 7 phylum and top 7 genera. The phylum *Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Actinobacteria, Tenericutes*, and *Verrucomicrobia* account for more than 95% of the healthy human gut microbes,[20] and the genera Bacteroides, *bifidobacterium*, *eubacterium, clostridium, peptococcus, peptostreptococcus*, and *ruminococcus* are predominant in human gut microbes,[21-23] so the differences between the two groups in those categories were summarized.

Table 2: Methodology and findings of the included studies assessing beta diversity for comparing the COVID-19 and healthy control groups.

Study	Metric	Analysis	Result
Silan Gu 2020	PCoA of Bray-Curtis	ANOSIM	Significant different
Mohammad Al Bataineh 2021	PCoA of weighted UniFrac		Significant different
Jiabao Cao 2021	PCoA of Bray-Curtis	PERMANOVA	Significant different
Paolo Gaibani 2021	PCoA of Bray-Curtis	Permutation test with pseudo-F ratio	Significant different
Sabine Hazan 2021	PCoA of weighted UniFrac		Significant different
Sijia Li 2021	PCoA of Bray-Curtis		Significant different
Zhigang Ren 2021	PCoA based on OTU distribution		Significant different
Yun Kit Yeoh 2021	PCoA	PERMANOVA	Significant different
Tuoyu Zhou 2021	PCoA of Bray-Curtis		Significant different
Zhonghan Sun 2022	PCoA of unweighted Unifrac	PERMANOVA	Significant different

The differences between healthy control and COVID-19 groups are shown in Figure 3.

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At the Phylum level, Firmicutes showed a trend of reduced relative abundance in the intestines of COVID-19 patients compared to healthy individuals. At the genus level, the relative abundance of *bifidobacterium*, *ruminococcus*, and *peptostreptococcus* is significantly reduced in COVID-19 patients in most of the included studies.

Discussion

Because of the strong infectious characteristics of SARS-CoV2, there is no large-scale population study on the intestinal flora of COVID-19 patients. The limited number of cases in each study and the choice of reference population may be an essential source of heterogeneity between studies. I performed alpha diversity analysis on 230 patients and 182 healthy controls through a systematic and comprehensive search of published research. The result (Shannon Index SMD: -0.81(-1.56, -0.06)) showed that the alpha diversity of intestinal flora decreased significantly in COVID-19 patients (Figure 2). The decrease of intestinal flora diversity may be an essential process of a series of pathological conditions caused by SARS-CoV-2 virus invasion.

The beta diversity analysis has shown that the components of the patient's gut flora have changed significantly compared to healthy controls (Table 2).

The relative abundance of specific phyla and genera in COVID-19 patients compared to healthy controls was also summarized (Figure 3). Due to the limited number of cases and the fact that not all phylum and genus were studied in each publication, the relevant abundance for the majority of the phylum and genus is not yet apparent, and more studies with an increased number of cases will be needed to obtain a solidified conclusion. However, the results show that the relevant abundance of the *ruminococcus* genus, *peptostreptococcus* genus, and the *bifidobacterium* genus was significantly reduced in COVID-19 patients. Bifidobacterium is an abundant type of health-promoting bacteria in the gut of healthy individuals that helps with digestion and has immunomodulatory functions.

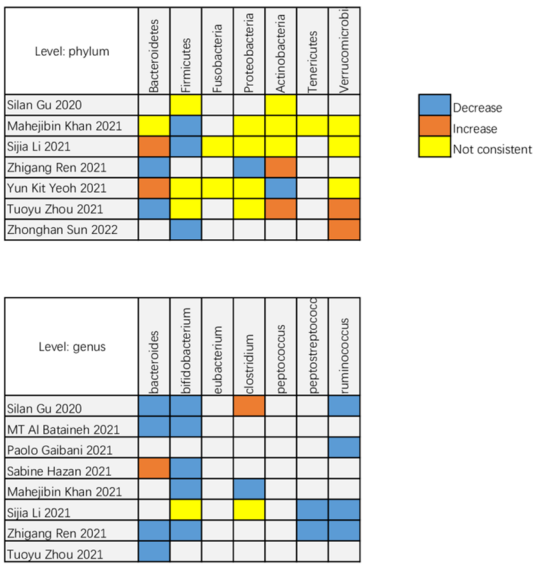

Figure 3: Compared to the healthy group, changes in the relative abundance of gut microbial taxa in the COVID-19 group.

To my knowledge, this is the first research to assess gut microbiota perturbations in COVID-19 patients through a systematic and comprehensive search and statistical analysis. As the gut microbiome is severely perturbed in COVID-19 patients, it can be used as a biomarker in monitoring patients' disease progress and recovery. Additionally, restoring the gut microbiome can be used as an adjuvant therapy to treat COVID-19 patients. COVID-19 can involve sequelae and a broad range of medical complications that last months after the initial recovery. Coincidently, the perturbation of the gut microbiome in COVID-19 patients can also last months after recovery. Since the gut microbiome plays a vital role in many host functions, including metabolism, strengthening gut integrity, and regulating host immunity, restoring the gut microbiome by supplementing patients with beneficial microorganisms may serve as a promising novel adjuvant therapy during the disease phase and post-acute phase to counteract long-lasting complications of COVID-19.

Conclusion

I performed a systematic and comprehensive search of published studies in this study and analyzed 11 selected studies for gut microbiome alpha diversity, beta diversity, and relative abundance indexes. The results suggest that COVID-19 patients are associated with aberrant microbiome composition, as reflected by a statistically significant decrease in microbial diversity compared to healthy individuals. In addition, COVID-19 patients exhibited decreased health-promoting bacteria, especially *Bifidobacterium*, which is abundant in healthy individuals. This study indicates that the gut microbiome can serve as a biomarker to monitor the disease progression and treatment effectiveness; that is, full recovery of a healthy microbiome is a valid indicator for the recovery of the patients. These findings also suggest that restoring the health-promoting bacteria may serve as a promising novel adjuvant therapy for COVID-19.

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References

1. https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
2. Proal A.D. and B. Vanelzakker. Long COVID or post-acute sequelae of COVID-19 (PASC): an overview of biological factors that may contribute to persistent symptoms. Frontiers in Microbiology, 2021, 12: 698169.
3. Desai A. D., M. Lavelle, B. C. Boursiquot, and E. Y. Wan. Long-term complications of COVID-19. Am J Physiol Cell Physiol 2022, 322: C1-C11.
4. Hamming, I.; Timens, W.; Bulthuis, M. L.; Lely, A. T.; Navis, G.; van Goor, H., Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004, 203 (2), 631-7.
5. Hashimoto, T.; Perlot, T.; Rehman, A.; Trichereau, J.; Ishiguro, H.; Paolino, M.; Sigl, V.; Hanada, T.; Hanada, R.; Lipinski, S.; Wild, B.; Camargo, S. M.; Singer, D.; Richter, A.; Kuba, K.; Fukamizu, A.; Schreiber, S.; Clevers, H.; Verrey, F.; Rosenstiel, P.; Penninger, J. M., ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 2012, 487 (7408), 477-81.

6. Hutton, B.; Salanti, G.; Caldwell, D. M.; Chaimani, A.; Schmid, C. H.; Cameron, C.; Ioannidis, J. P.; Straus, S.; Thorlund, K.; Jansen, J. P.; Mulrow, C.; Catalá-López, F.; Gøtzsche, P. C.; Dickersin, K.; Boutron, I.; Altman, D. G.; Moher, D., The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. Ann Intern Med 2015, 162 (11), 777-84.
7. Ait Chait, Y.; Mottawea, W.; Tompkins, T. A.; Hammami, R., Unravelling the antimicrobial action of antidepressants on gut commensal microbes. Sci Rep 2020, 10 (1), 17878.
8. Simpson, C. A.; Diaz-Arteche, C.; Eliby, D.; Schwartz, O. S.; Simmons, J. G.; Cowan, C. S. M., The gut microbiota in anxiety and depression - A systematic review. Clin Psychol Rev 2021, 83, 101943.
9. Gu, S.; Chen, Y.; Wu, Z.; Chen, Y.; Gao, H.; Lv, L.; Guo, F.; Zhang, X.; Luo, R.; Huang, C.; Lu, H.; Zheng, B.; Zhang, J.; Yan, R.; Zhang, H.; Jiang, H.; Xu, Q.; Guo, J.; Gong, Y.; Tang, L.; Li, L., Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza. Clinical Infectious Diseases 2020, 71 (10), 2669-2678.
10. Al Bataineh, M. T.; Henschel, A.; Mousa, M.; Daou, M.; Waasia, F.; Kannout, H.; Khalili, M.; Kayasseh, M. A.; Alkhajeh, A.; Uddin, M.; Alkaabi, N.; Tay, G. K.; Feng, S. F.; Yousef, A. F.; Alsafar, H. S., Gut Microbiota Interplay With COVID-19 Reveals Links to Host Lipid Metabolism Among Middle Eastern Populations. Frontiers in Microbiology 2021, 12.
11. Cao, J. B.; Wang, C.; Zhang, Y. Q.; Lei, G. L.; Xu, K.; Zhao, N.; Lu, J. J.; Meng, F. P.; Yu, L. X.; Yan, J.; Bai, C. Q.; Zhang, S. G.; Zhang, N.; Gong, Y. H.; Bi, Y. H.; Shi, Y.; Chen, Z.; Dai, L. P.; Wang, J.; Yang, P. H., Integrated gut virome and bacteriome dynamics in COVID-19 patients. Gut Microbes 2021, 13 (1), 1-21.
12. Gaibani, P.; D'Amico, F.; Bartoletti, M.; Lombardo, D.; Rampelli, S.; Fornaro, G.; Coladonato, S.; Siniscalchi, A.; Re, M. C.; Viale, P.; Brigidi, P.; Turroni, S.; Giannella, M., The Gut Microbiota of Critically Ill Patients With COVID-19. Frontiers in Cellular and Infection Microbiology 2021, 11.
13. Hazan, S.; Stollman, N.; Bozkurt, H.; Dave, S.; Papoutsis, A. J.; Daniels, J.; Dolai, S.; Barrows, B. D.; Quigley, E. M. M.; Borody, T. J., The lost microbes of COVID-19: Bifidobacteria depletion and decreased microbiome diversity are a predictability marker of severe COVID 19, a cross sectional study. 2021.
14. Khan, M.; Mathew, B. J.; Gupta, P.; Garg, G.; Khadanga, S.; Vyas, A. K.; Singh, A. K., Gut Dysbiosis and IL-21 Response in Patients with Severe COVID-19. Microorganisms 2021, 9 (6), 16.
15. Li, S.; Yang, S.; Zhou, Y.; Disoma, C.; Dong, Z.; Du, A.; Zhang, Y.; Chen, Y.; Huang, W.; Chen, J.; Song, D.; Chen, Z.; Liu, P.; Li, S.; Zheng, R.; Liu, S.; Razzaq, A.; Chen, X.; Tao, S.; Yu, C.; Feng, T.; Liao, W.; Peng, Y.; Jiang, T.; Huang, J.; Wu, W.; Hu, L.; Wang, L.; Li, S.; Xia, Z., Microbiome Profiling Using Shotgun Metagenomic Sequencing Identified Unique Microorganisms in COVID-19 Patients With Altered Gut Microbiota. Frontiers in Microbiology 2021, 12.
16. Ren, Z.; Wang, H.; Cui, G.; Lu, H.; Wang, L.; Luo, H.; Chen, X.; Ren, H.; Sun, R.; Liu, W.; Liu, X.; Liu, C.; Li, A.; Wang, X.; Rao, B.; Yuan, C.; Zhang, H.; Sun, J.; Chen, X.; Li, B.; Hu, C.; Wu, Z.; Yu, Z.; Yu, Z.; Kan, Q.; Li, L., Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. Gut 2021, 70 (7), 1253-1265..
17. Yeoh, Y. K.; Zuo, T.; Lui, G. C. Y.; Zhang, F.; Liu, Q.; Li, A. Y. L.; Chung, A. C. K.; Cheung, C. P.; Tso, E. Y. K.; Fung, K. S.C.; Chan, V.; Ling, L.; Joynt, G.; Hui, D. S. C.; Chow, K. M.; Ng, S. S. S.; Li, T. C. M.; Ng, R. W. Y.; Yip, T. C. F.; Wong, G. L. H.; Chan, F. K. L.; Wong, C. K.; Chan, P. K. S.; Ng, S.
18. Zhou, T.; Zeng, Y.; Wu, J.; Li, J.; Yan, J.; Meng, W.; Han, H.; Feng, F.; He, J.; Zhao, S.; Zhou, P.; Wu, Y.; Yang, Y.; Han, R.; Jin, W.; Li, X.; Yang, Y.; Li, X., SARS-CoV-2 triggered excessive inflammation and abnormal energy metabolism in gut microbiota. 2021.
19. Sun, Z.; Song, Z. G.; Liu, C.; Tan, S.; Lin, S.; Zhu, J.; Dai, F. H.; Gao, J.; She, J. L.; Mei, Z.; Lou, T.; Zheng, J. J.; Liu, Y.; He, J.; Zheng, Y.; Ding, C.; Qian, F.; Zheng, Y.; Chen, Y. M., Gut microbiome alterations and gut barrier dysfunction are associated with host immune homeostasis in COVID-19 patients. BMC Medicine 2022, 20 (1).
20. Eckburg, P. B.; Bik, E. M.; Bernstein, C. N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S. R.; Nelson, K. E.; Relman, D. A., Diversity of the human intestinal microbial flora. Science 2005, 308 (5728), 1635-8.
21. Bengmark, S., Ecological control of the gastrointestinal tract. The role of probiotic flora. Gut 1998, 42 (1), 2-7.
22. Guarner, F.; Malagelada, J. R., Gut flora in health and disease. Lancet 2003, 361 (9356), 512-9.
23. Simon, G. L.; Gorbach, S. L., Intestinal flora in health and disease. Gastroenterology 1984, 86 (1), 174-93.

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