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Full Paper

Differences in the human gut microbiota with varying depressive symptom severity scores

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Depression is a prevalent mental health disorder, and its incidence has increased further because of the coronavirus disease 2019 (COVID-19) pandemic. The gut microbiome has been suggested as a potential target for mental health treatment because of the bidirectional communication system between the brain and gastrointestinal tract, known as the gut-brain axis. We aimed to investigate the relationship between the human gut microbiome and depression screening by analyzing the abundance and types of microbiomes among individuals living in Japan, where mental health awareness and support may differ from those in other countries owing to cultural factors. We used a data-driven approach to evaluate the gut microbiome of participants who underwent commercial gut microbiota testing services and completed a questionnaire survey that included a test for scoring depressive tendencies. Our data analysis results indicated that no significant differences in gut microbiome composition were found among the groups based on their depression screening scores. However, the results also indicated the potential existence of a few differentially abundant bacterial taxa. Specifically, the detected bacterial changes in abundance suggest that the Bifidobacteriaceae, Streptococcaceae, and Veillonellaceae families are candidates for differentially abundant bacteria. Our findings should contribute to the growing body of research on the relationship between gut microbiome and mental health, highlighting the potential of microbiome-based interventions for depression treatment. The limitations of this study include the lack of clear medical information on the participants' diagnoses. Future research could benefit from a larger sample size and more detailed clinical information.

Key words: gut microbiota, mental health, 16s rRNA, direct-to-consumer Mykinso gut-microbiome testing service

INTRODUCTION

In modern society, mental health is a relevant part of lifestyle for many people. According to the World Health Organization, depression is very prevalent worldwide; approximately 5.0% of adults suffer from depression, and it is the leading cause of disability worldwide [1]. Since the emergence of the coronavirus disease 2019 (COVID-19) pandemic, stress has become a global concern, especially because of the change in or lack of social interactions due to protective measures against viral infection. This social shift may have caused drastic economic and social stress, resulting in the ubiquity of depression and anxiety. The World Health Organization underscores the need for action on mental health during the COVID-19 pandemic [2].

One possible solution to this global crisis is to investigate the relationship between mental health and the gut microbiome. Many studies on the human gut microbiome have been conducted recently because it has become easier to analyze the taxonomy of enteric bacteria using DNA sequencing techniques such as high-throughput sequencing, which enables extensive categorization

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of metagenomic samples [3]. Some recent research projects have found intriguing correlations between the gut microbiome and health. One of these correlations is known as the gut-brain axis, which is a bidirectional communication system that links the brain and its cognitive and emotional function with the gastrointestinal tract [4].

Studies have already been conducted on the gut-brain axis and the involvement of gut microbiota in host health, and they have shown mixed results and conclusions [5]. Jiang *et al.* and Zheng *et al.* reported differences in bacterial composition in healthy control (HC) patients and patients with major depressive disorder (MDD) [6, 7]. In contrast, Naseribafrouei *et al.* reported no differences between HC and MDD bacterial taxa [8]. In experiments, including theirs, some potential bacterial factors that distinguish healthy from mentally unhealthy individuals have been suggested, but no clear treatments have been developed in previous studies.

Studies have demonstrated that in Japan, mental health may not be dealt with properly because of the cultural tendency of people to hold onto their feelings and try to solve problems on their own, instead of sharing problems with others. Kanehara *et al.* conducted an interview survey among random individuals in 11 communities across Japan between 2002 and 2006 [9]. They found that more than half of the individuals did not seek mental health care or dropped out of it because of low perceived needs. Although the need for and significance of mental health awareness and support is high, the Japanese community may also have a different background in its microbial characteristics. A previous study showed the uniqueness of the gut microbiome of healthy individuals in Japan [10]. However, only a few studies have examined the relationship between the microbiome and mental health in Japanese communities, so Japan could benefit from more research on the topic.

Despite the need, such research is challenging to conduct. In gut microbiome research, it is important to collect a sufficient number of samples to statistically determine the variable of interest [11, 12]. However, collecting samples is often difficult when attempting to identify the gut bacteria associated with a disease or a clinical phenotype. To collect a sufficient number of samples that differ only with regard to a specific disease and are matched in terms of other attributes, such as age, gender, and race, it may be necessary to conduct a large project such as a cohort study. Previous studies that have been conducted on the correlation between mental health and gut microbiota have also had a limited number of samples [5].

Therefore, we attempted to ensure a sufficient sample size using data from commercially available gut microbiota-testing services. Unlike data obtained from healthcare facilities, no information on the diagnosis of the disease by a doctor is available when using such data, and it is not possible to categorize samples according to the disease. However, the service conducted a questionnaire survey of the participants, which included a test for scoring depressive tendencies. In exchange for the lack of clear medical information, the large sample size provided by the service enables data-driven research.

In this study, we evaluated differences in the abundance and types of microbiomes among people living in Japan, grouped by their mental health conditions. To identify the bacterial factors that influence human mental health through the study of gut bacteria, we evaluated health conditions and habits using questionnaires.

MATERIALS AND METHODS

Sample collection and filtering criteria

The study initially included a total of 8,340 fecal samples derived from 16S rRNA sequencing data, which were registered in the Mykinso cohort from November 2016 to October 2020. The Mykinso cohort is one of the largest gut microbiota research cohorts in Japan and is composed of the microbiota profiles and demographic and lifestyle habit survey data of individuals who consented to participate in research among the users of Mykinso, a direct-to-consumer gut flora testing service. The study was approved by the Cykinso Research Ethics Committee (No. LD-001-04 and LD-002-03). All procedures complied with the principles of the Declaration of Helsinki and were approved by the Institutional Review Board (IRB) at our institution, and the study was registered under UMIN000028887 and UMIN000028888 in the UMIN Clinical Trials Registry System. The IRB-approved protocol specifically allows for a study involving a cross-sectional (one time per subject) analysis of survey data and subsequent follow-up surveys (multiple times per subject). In this study, we only analyzed cross-sectional data from the cohort study dataset. The protocols for fecal sampling, DNA extraction, and sequencing have been described in our previous article [13].

The participants of the cohort study answered a questionnaire on lifestyle and daily habits. The questionnaire included questions asking the participants about their mental conditions at the time of sampling, which followed the Center for Epidemiological Studies Depression (CES-D) scale protocol, a standard depression measurement [14]. We collected 3,782 samples with answers that led to valid CES-D score calculations. To eliminate the effects of other diseases that may affect the gut microbiome as much as possible, we filtered the samples using the answers to the questions listed in Table 1. The questions related to diseases, drugs, and irregular state of stools were picked from the questionnaire. Samples were used for further analysis when all the answers to those questions were “No”. A total of 472 samples that met these requirements were used for further analyses. The collected samples were grouped according to their calculated CES-D scores. The CES-D score ranges from 0 to 60, and individuals with a score equal to or more than 16 are considered to have depression tendencies in recommended use cases. Hence, we performed a comparison between two sample groups (2-group comparison): one group with CES-D scores lower than 16 (low) and another group with scores equal to or higher than 16 (high). However, it is important to acknowledge the recognized limitations of this scale as highlighted in a past study [15]. Given that depressive symptoms exist on a spectrum rather than a binary state, we tried to establish an intermediate state within the CES-D score, which we set at 5 points above and below the recommended threshold. Consequently, we undertook an additional comparison (3-group comparison), categorizing the collected samples into low (<11), medium (>10 and <20), and high (>20) groups.

Sequencing data processing and visualization

Raw 16S rRNA sequencing data processing included demultiplexing, denoising, taxonomy assignment, and beta diversity analysis. We performed these steps using QIIME 2 (version 2020.2) [16]. The classifier was trained with a robust taxonomy simplifier for SILVA (version 138) [17]. We conducted

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these steps using a workflow composed of the Common Workflow Language (CWL), a specification for describing data analysis workflows [18]. The CWL workflow definition files, including the executed commands and used parameters, are available from our GitHub repository (https://github.com/pitagora-network/DAT2-cwl/tree/main/workflow/meta16s-seq). Differential abundance analysis was performed using the ANCOM-BC Bioconductor package [19]. The software setup and analysis procedures were performed and recorded in Jupyter Notebook [20]. We also used the Jupyter Notebook to visualize the results of the analysis.

RESULTS

Sample attribute distribution

Table 2 provides a comprehensive overview of the characteristics of the study participants, both collectively and categorized by CES-D score. The total participant count was 457, with a mean age of 41.33 ± 10.37. Supplementary Fig. 1 shows the age distribution for each group of participants. Additionally, Table 2 presents statistics for age, sex, body mass index (BMI), smoking habit, and CES-D score for both the overall participant pool and the groups analyzed in the two comparisons—2 groups and 3 groups. Minimal discrepancies were observed in sex, BMI, or smoking habits between the groups, minimizing potential biases in subsequent analyses. Figure 1 depicts the distribution of CES-D scores for all participants, demonstrating a distribution relatively consistent with previous studies on a Japanese population with a higher mean age ± SD of 62.0 ± 10.36 compared with our study participants [21].

Beta-diversity analysis

To assess the beta diversity of bacterial species across different groups, we conducted a principal coordinate analysis (PCoA) using the EMPeror plugin within the QIIME 2 toolkit [22]. The outcomes of both the 2-group and 3-group comparisons revealed no discernible differences in bacterial compositions among the groups. Figure 2 displays the results based on Bray–Curtis dissimilarity metrics from the 3-group comparison, while Supplementary Fig. 2 presents outcomes obtained using alternative distance methods for PCoA analysis, which all indicated similar compositions among the groups. These findings suggest that the CES-D score has a limited impact on the overall bacterial composition in the human gut microbiome.

Differential abundance analysis

To identify potential taxa exhibiting differential abundance among bacteria, we employed the ANCOM-BC program. The program provides outputs including p-values, q-values, and W statistics. In the ANCOM-BC analysis, both the p-value and q-value for a taxon can be zeros, which are termed “structural zeros”, indicating the taxon’s absence in at least one sample group. W signifies the count of sub-hypotheses passed by ANCOM-BC for the taxon—a higher W value indicates a greater likelihood of being differentially abundant, though the value can be negative. In Table 3, the negative W values indicate a reduction in taxa abundance in the low group (CES-D score <16) compared with the reference (group: high).

Table 3 presents the taxa from the 2-group comparison with non-zero p-values, identified as “differentially abundant among the groups” by the software, while Supplementary Table 1 shows the structural zeros found in the 2-group and 3-group comparisons.

Table 1. List of questions used for sample filtering

Questions
Do you have any chronic or pre-existing medical conditions?
Have you been treated for *Helicobacter pylori* before?
Have you been diagnosed with colorectal cancer/colorectal polyps in the last 5 years?
Have you been diagnosed with bacterial enteritis in the last 3 months?
Are you currently taking any medicines?
Have you been using supplements regularly in the last month or more?
Have you had muddy or watery stools in the last 3 months without taking laxatives?
Do you think you have been constipated in the last 3 months?
Have you taken any antibiotics in the last 3 months before the stool collection?
Is BMI equal or lower than 30?

BMI: body mass index.

Table 2. Summary of statistics for all samples and each group

	CES-D score range	Total number of samples	Age (mean ± SD)	Female/ Male	BMI (mean ± SD)	Smoking/ Non smoking	CES-D score (mean ± SD)
Total	0–60	457	41.33 ± 10.37	228 / 229	21.61 ± 2.86	28 / 429	8.81 ± 7.19
2 Groups: Low	0–15	393	42.07 ± 10.41	190 / 203	21.69 ± 2.87	22 / 371	6.62 ± 4.30
2 Groups: High	16–60	64	36.75 ± 8.96	38 / 26	21.08 ± 2.76	6 / 58	22.28 ± 6.72
3 Groups: Low	0–10	302	42.06 ± 10.27	147 / 155	21.66 ± 2.94	14 / 288	4.84 ± 3.12
3 Groups: Middle	11–20	128	40.53 ± 10.73	65 / 63	21.57 ± 2.65	12 / 116	14.12 ± 2.86
3 Groups: High	21–60	27	36.89 ± 8.59	16 / 11	21.22 ± 2.91	2 / 25	28.15 ± 6.63

CES-D: Center for Epidemiological Studies Depression; BMI: body mass index; SD: standard deviation.

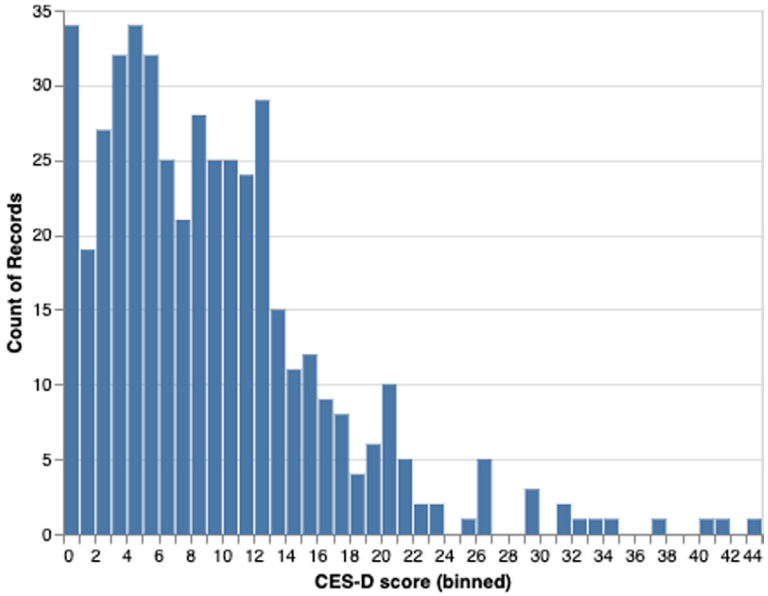

Fig. 1. The distribution of participants' CES-D scores.

The score range was 0–44, and the mean score with SD was 8.81 ± 7.19, while the score with SD from the past study of the Japanese population was 10.9 ± 6.63. CES-D: Center for Epidemiological Studies Depression; SD: standard deviation

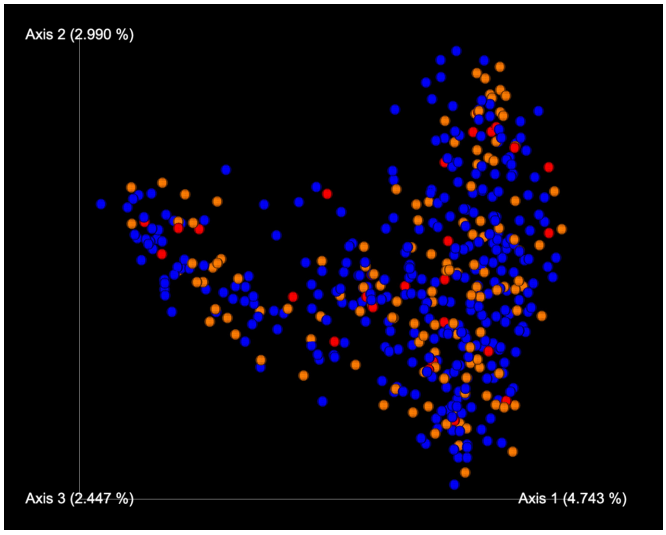

Fig. 2. The visualization of PCoA analysis based on Bray Curtis dissimilarity metrics.

The distribution of the colored dots shows no clear differences between these groups. The red dots are high, oranges are middle, and blues are low. PCoA: principal coordinate analysis.

Notably, ANCOM-BC found only taxa with structural zeros in the 3-group comparison, which may have resulted from the smaller number of samples in the CES-D high group.

To visualize the distribution of abundance ratios of the differentially abundant taxa for each sample in the 2-group comparison, we utilized Jitter plots for those taxa (Fig. 3). As depicted in the plot, among the 5 families with differential abundances, three of them, Bifidobacteriaceae, Streptococcaceae, and Veillonellaceae, were observed to have lower abundances in the low CES-D score group. Although there was a difference in the numbers of samples (low, 393; high, 64), these taxa could be those of interest in further research.

DISCUSSION

In this study, we observed that participants divided into both 2 groups (± 16) and 3 groups (<10, 10–20, 21≤) based on their CES-D scores exhibited similar compositions of gut bacteria. However, the results of the differential abundance analysis by ANCOM-BC suggested potential variations in the abundances of certain bacteria among the groups. It is important to note that the statistical significance of these findings needs to be established, necessitating further research.

Our investigation into the microbial composition among individuals with varying CES-D scores has unveiled intriguing findings regarding specific bacterial families. We observed differences in the abundance of certain bacterial families between healthy individuals and those exhibiting higher CES-D scores, indicative of heightened tendencies towards depression. Specifically, we identified elevated levels of the Bifidobacteriaceae, Streptococcaceae, and Veillonellaceae families in individuals with higher CES-D scores, suggesting a potential association between these microbial populations and depressive symptomatology.

However, these findings do not always align with existing research, providing an interesting context for our observations. For instance, a study by Aizawa *et al.* highlighted significantly lower counts of *Bifidobacterium* among patients diagnosed with MDD, which contradicts our data showing an increased abundance of Bifidobacteriaceae in individuals with higher CES-D scores [23]. Furthermore, the link between probiotics containing bifidobacteria and their potential therapeutic effects on mental health, as elucidated in Li *et al.*, may be of interest

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Table 3. Results of ANCOM-BC for each taxonomic level showing non-zero p-values, from the 2-group comparison (CES-D score lower/higher than 16)

Phylum	Class	Order	Family	Genus	Species	W	p_val	q_val
Actinobacteriota						−3.916528	8.98E-05	0.000808501
Actinobacteriota	*Actinobacteria*					−3.945068	7.98E-05	0.001116884
Actinobacteriota	*Actinobacteria*	*Actinomycetales*				−3.425771	0.000613058	0.01777868
Actinobacteriota	*Actinobacteria*	*Actinomycetales*	*Actinomycetaceae*			−4.340077	1.42E-05	0.000697921
Actinobacteriota	*Actinobacteria*	*Bifidobacteriales*				−3.172003	0.001513917	0.04238967
Actinobacteriota	*Actinobacteria*	*Bifidobacteriales*	*Bifidobacteriaceae*			−3.880309	1.04E-04	0.005007534
Actinobacteriota	*Coriobacteriia*	*Coriobacteriales*	*Eggerthellaceae*			−3.803707	1.43E-04	0.00669971
Firmicutes	*Bacilli*	*Lactobacillales*	*Streptococcaceae*			−3.417855	6.31E-04	0.028402498
Firmicutes	*Negativicutes*	*Veillonellales-Selenomonadales*	*Veillonellaceae*			−3.725926	1.95E-04	0.008951581
Fusobacteriota						2.933003	3.36E-03	0.026856034

CES-D: Center for Epidemiological Studies Depression.

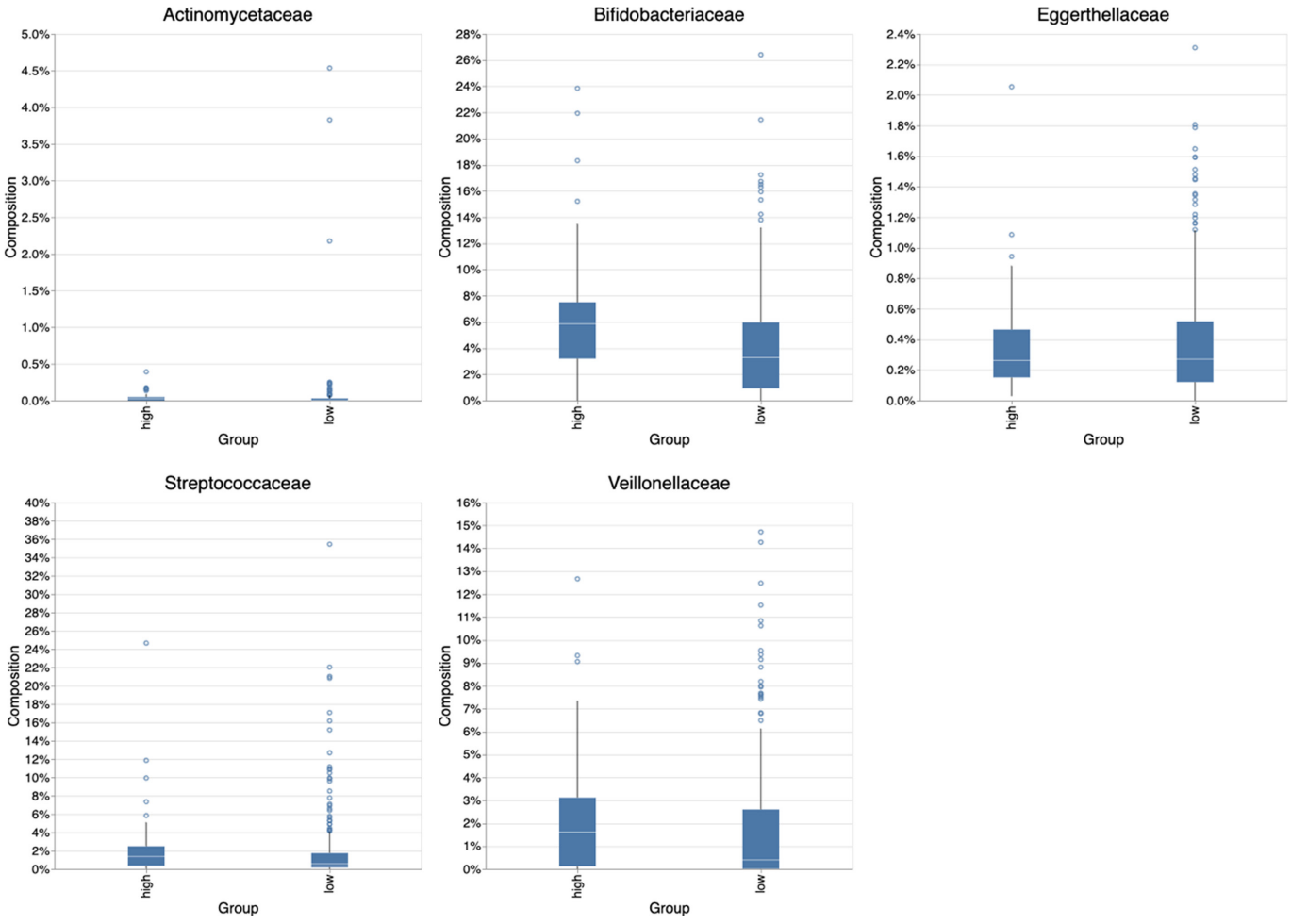

Fig. 3. Jitter plots of the distribution of abundance ratios for selected taxa per group that showed non-zero p-values in the 2 groups comparison.
Each circle indicates a sample included in the group with the abundance ratio of the taxa in percentage. Although not all the samples contained the taxa, these had a different range of abundance ratios between the groups.

given the relevance of our data in the context of emerging treatment modalities for depression and related disorders [24]. The work by Jiang *et al.* also highlighted a decrease in Veillonellaceae abundance among MDD samples and an increase in Veillonellaceae *Megamonas* among active MDD (A-MDD) samples, which also contradicts our results [6]. On the other hand, investigations into the role of *Streptococcus* in MDD further support our results. Lin *et al.* reported an increase in the abundance of *Streptococcus* among MDD samples, consistent with our observation of heightened levels of Streptococcaceae in individuals with higher CES-D scores [25].

In our study, we utilized samples obtained from a genetic testing service, categorizing the data based on CES-D scores derived from a questionnaire. The advantage of employing these samples lies

in the substantial number of participants they represent. However, a drawback is that our investigation focused on individuals lacking a clinical diagnosis of depression. Consequently, any findings from this study should not be extrapolated to the nature of depression as a clinical condition. Nevertheless, the results may offer valuable insights for future studies on depression. Despite the comparatively large sample size in our study, the high CES-D score group had a limited number of samples. To address potential bias stemming from uneven sample distribution across groups, we employed ANCOM-BC. It is essential to note that the results might be subject to change with the inclusion of more samples with higher CES-D scores.

The results of our study and the form of the investigation open up new applications that allow us to achieve a better understanding and improvement of an individual's mental health. One example of this could be the creation of a system that tracks the health of the same individual over a long period to ensure that they have a healthy gut condition. This could evolve into an alert and monitoring system using the CES-D score with the gut microbiome, possibly focusing more on fecal samples for the diagnosis of MDD in the future.

Furthermore, studies have also suggested the possibility of treating people with dietary changes or fecal transplants to improve their mental health [4]. Further research could also explore the potential of using probiotics and prebiotics to modulate the gut microbiome and improve mental health outcomes. These interventions could offer a safe and natural alternative or complementary treatment for individuals with mental health conditions, particularly those who experience adverse effects from traditional pharmacological therapies.

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CONFLICT OF INTEREST

None.

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