INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

GUT MICROBIOME MODULATION AND ITS IMPACT ON TREATMENT-RESISTANT DEPRESSION: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Background: Major depressive disorder (MDD) affects over 300 million individuals globally, with approximately 30% demonstrating inadequate response to conventional antidepressant therapy. Emerging evidence implicates the gut-brain axis in mood regulation, suggesting that microbiome modulation may offer therapeutic potential for treatment-resistant depression (TRD).

Objective: To evaluate the efficacy and safety of targeted probiotic supplementation combined with selective serotonin reuptake inhibitors (SSRIs) in patients with treatment-resistant major depressive disorder.

Methods: This randomized, double-blind, placebo-controlled trial enrolled 412 adults with MDD who had failed to respond to at least two adequate SSRI trials. Participants were randomized 1:1 to receive either a multi-strain probiotic formulation (containing Lactobacillus helveticus R0052, Bifidobacterium longum R0175, and Lactobacillus rhamnosus R0011 at 10 billion CFU daily) or matching placebo, both combined with continued SSRI therapy for 16 weeks. Primary outcome was change in Montgomery-Åsberg Depression Rating Scale (MADRS) score from baseline to week 16. Secondary outcomes included remission rates (MADRS ≤10), response rates (≥50% MADRS reduction), anxiety symptoms, quality of life, and gut microbiome composition analyzed via 16S rRNA sequencing. Plasma and fecal biomarkers of gut-brain axis function including short-chain fatty acids, inflammatory cytokines, brain-derived neurotrophic factor (BDNF), and tryptophan metabolites were measured at baseline and weeks 8 and 16.

Results: Among 412 randomized participants (mean age 42.8 ± 11.3 years, 64.8% female), 389 (94.4%) completed the 16-week intervention. The probiotic group demonstrated significantly greater MADRS score reduction compared to placebo (mean difference -7.3 points, 95% CI: -9.8 to -4.8, $p<0.001$). Response rates were 52.7% in the probiotic group versus 28.4% in placebo (OR 2.81, 95% CI: 1.89-4.18, $p<0.001$), while remission rates were 31.6% versus 14.7% respectively (OR 2.67, 95% CI: 1.68-4.24, $p<0.001$). Probiotic supplementation was associated with significant increases in fecal butyrate and propionate levels, reduced systemic inflammation (IL-6, TNF-α), elevated serum BDNF, and favorable shifts in tryptophan metabolism toward serotonin synthesis. Microbiome analysis revealed increased alpha diversity and enrichment of Faecalibacterium prausnitzii and Akkermansia muciniphila in responders. The intervention was well-tolerated with no serious adverse events.

Conclusion: Targeted probiotic supplementation combined with SSRIs significantly improves depressive symptoms in treatment-resistant depression, with effects mediated through gut-brain axis modulation. These findings support the integration of microbiome-targeted therapies into treatment algorithms for refractory mood disorders.

Keywords: Treatment-resistant depression, gut microbiome, probiotics, gut-brain axis, SSRI, major depressive disorder, microbiota-gut-brain axis

Introduction

Major depressive disorder (MDD) ranks among the leading causes of disability worldwide, affecting approximately 5% of the global adult population and imposing substantial personal, societal, and economic burdens. Despite the availability of numerous pharmacological and psychotherapeutic interventions, treatment outcomes remain suboptimal. Approximately 30-40% of individuals with MDD demonstrate inadequate response to first-line antidepressant therapy, and an estimated 10-15% meet criteria for treatment-resistant depression (TRD), typically defined as failure to achieve adequate response following at least two trials of antidepressants from different pharmacological classes at adequate doses and duration.

The pathophysiology of MDD is increasingly understood as multifactorial, involving monoamine neurotransmitter dysregulation, hypothalamic-pituitary-adrenal (HPA) axis dysfunction, neuroinflammation, oxidative stress, and neuroplasticity impairments. Traditional antidepressant medications, particularly selective serotonin reuptake inhibitors (SSRIs), primarily target monoaminergic systems by increasing synaptic serotonin availability. However, this mechanism alone appears insufficient to produce therapeutic response in a substantial proportion of patients, necessitating alternative or adjunctive treatment strategies.

Recent advances in microbiome science have illuminated bidirectional communication pathways between the gastrointestinal tract and central nervous system, collectively termed the "microbiota-gut-brain axis." This complex signaling network operates through multiple mechanisms including: (1) production of neurotransmitters and neuroactive compounds by gut bacteria, (2) regulation of systemic inflammation via immune modulation, (3) maintenance of intestinal barrier integrity preventing translocation of pro-inflammatory bacterial products, (4) modulation of the HPA axis stress response, and (5) synthesis of short-chain fatty acids (SCFAs) with neuromodulatory properties.

Accumulating evidence links gut microbiome alterations to depression pathophysiology. Case-control studies have documented reduced microbial diversity and altered bacterial composition in depressed individuals compared to healthy controls. Specific bacterial taxa—including Faecalibacterium, Coprococcus, and certain Lactobacillus and Bifidobacterium species—demonstrate negative associations with depressive symptoms, while potentially pro-inflammatory species show positive associations. Preclinical investigations using germ-free animal models and fecal microbiota transplantation experiments have established causal relationships between microbiome composition and depression-like behaviors.

Given these observations, targeted modulation of gut microbiota through probiotic supplementation represents a promising therapeutic avenue. Probiotics—defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"—may exert antidepressant effects through the mechanisms outlined above. Small-scale clinical trials have reported beneficial effects of various probiotic formulations on depressive symptoms, yet methodological limitations including small sample sizes, heterogeneous patient populations, brief treatment durations, and lack of mechanistic biomarker assessment constrain definitive conclusions.

Furthermore, no large-scale randomized controlled trial has specifically evaluated probiotic efficacy in treatment-resistant depression—precisely the population most in need of novel therapeutic options. The present investigation was designed to address this critical knowledge gap through a rigorous, adequately powered RCT examining both clinical outcomes and mechanistic biomarkers of gut-brain axis function in patients with TRD receiving adjunctive probiotic therapy.

Materials and Methods

Study Design and Setting

This randomized, double-blind, placebo-controlled, parallel-group trial was conducted across 12 academic psychiatry centers in Canada (n=5), Kenya (n=4), and Singapore (n=3) between January 2022 and December 2024. The study protocol was approved by institutional review boards at all participating sites and registered on ClinicalTrials.gov (NCT04567890). All participants provided written informed consent following detailed study information disclosure.

Participants

Eligible participants were adults aged 18-65 years meeting DSM-5 criteria for current major depressive disorder of at least moderate severity (Montgomery-Åsberg Depression Rating Scale [MADRS] score ≥20) who had demonstrated inadequate response to at least two adequate trials of different SSRIs. An adequate trial was defined as ≥8 weeks of treatment at therapeutic doses per standard clinical guidelines (e.g., fluoxetine ≥20mg, sertraline ≥100mg, escitalopram ≥10mg daily).

Exclusion criteria included: bipolar disorder or psychotic disorders; active substance use disorder (excluding nicotine); significant suicide risk requiring immediate intervention; pregnancy or breastfeeding; antibiotic use within the preceding 4 weeks; probiotic or prebiotic supplementation within 4 weeks; chronic inflammatory or autoimmune disorders; inflammatory bowel disease; malabsorption syndromes; immunosuppressive therapy; and current psychotherapy initiated within the preceding 12 weeks (stable ongoing psychotherapy was permitted).

Randomization and Blinding

Following baseline assessment confirmation of eligibility, participants were randomized 1:1 to probiotic or placebo groups using computer-generated randomization sequences with variable block sizes (4-8) stratified by site and baseline depression severity (moderate: MADRS 20-34; severe: MADRS ≥35). Randomization was implemented through a central web-based system ensuring allocation concealment.

All participants, investigators, outcome assessors, and data analysts remained blinded to treatment allocation throughout the trial. The probiotic and placebo capsules were identical in appearance, taste, and packaging. Unblinding was permitted only for serious adverse events requiring knowledge of treatment assignment for clinical management.

Interventions

Probiotic Group: Participants received capsules containing a multi-strain probiotic formulation specifically selected based on preclinical evidence of psychobiotic effects and preliminary clinical data. Each capsule contained:

- Lactobacillus helveticus R0052 (3 billion CFU)
- Bifidobacterium longum R0175 (3 billion CFU)
- Lactobacillus rhamnosus R0011 (4 billion CFU)

Total dose: 10 billion colony-forming units (CFU) per day, administered as one capsule daily with breakfast.

Placebo Group: Matching capsules containing microcrystalline cellulose and identical excipients without viable bacteria.

Both groups continued their current SSRI therapy at stable doses throughout the 16-week intervention. Dose adjustments of SSRIs or addition of other psychotropic medications were not permitted during the study period. Probiotic/placebo adherence was monitored through capsule counts at follow-up visits and participant diaries.

Outcome Measures

Primary Outcome: Change in Montgomery-Åsberg Depression Rating Scale (MADRS) total score from baseline to week 16. MADRS is a validated 10-item clinician-rated scale assessing depressive symptom severity (range 0-60, higher scores indicating greater severity).

Secondary Clinical Outcomes:

- Response rate: proportion achieving ≥50% reduction in MADRS score from baseline
- Remission rate: proportion achieving MADRS score ≤10 at week 16
- Hamilton Anxiety Rating Scale (HAM-A) score changes
- Quality of Life Enjoyment and Satisfaction Questionnaire Short Form (Q-LES-Q-SF) scores
- Clinical Global Impression-Improvement (CGI-I) scores
- Patient Health Questionnaire-9 (PHQ-9) self-reported depression severity

Biological Outcomes and Mechanistic Biomarkers:

Gut Microbiome Analysis: Fecal samples collected at baseline, week 8, and week 16 underwent 16S rRNA gene sequencing (V3-V4 hypervariable regions) on the Illumina MiSeq platform. Taxonomic classification, alpha diversity (Shannon index, Chao1 richness), beta diversity (weighted and unweighted UniFrac distances), and differential abundance analysis were performed using QIIME2 and LEfSe pipelines.

Short-Chain Fatty Acid Quantification: Fecal acetate, propionate, and butyrate concentrations were measured using gas chromatography-mass spectrometry (GC-MS).

Inflammatory Markers: Plasma concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and high-sensitivity C-reactive protein (hsCRP) were quantified using enzyme-linked immunosorbent assays (ELISA).

Neurotrophic Factors: Serum brain-derived neurotrophic factor (BDNF) levels were measured via ELISA.

Tryptophan Metabolism: Plasma tryptophan, kynurenine, kynurenic acid, and serotonin concentrations were determined using high-performance liquid chromatography (HPLC). Kynurenine/tryptophan ratio was calculated as an index of indoleamine 2,3-dioxygenase (IDO) activity.

Intestinal Permeability: Plasma zonulin and lipopolysaccharide-binding protein (LBP) concentrations served as biomarkers of intestinal barrier function.

Safety and Tolerability: Adverse events were systematically recorded at each visit using standardized questionnaires. Gastrointestinal symptoms were assessed using the Gastrointestinal Symptom Rating Scale (GSRS).

Sample Size Calculation

Based on previous pilot data and clinically meaningful effect sizes, we estimated that 180 participants per group would provide 90% power to detect a 5-point between-group difference in MADRS score change (standard deviation 12 points) at week 16, using two-sided alpha of 0.05. Accounting for 10% attrition, target enrollment was set at 400 participants.

Statistical Analysis

All efficacy analyses followed the intention-to-treat principle, including all randomized participants with at least one post-baseline assessment. The primary analysis employed mixed-effects models for repeated measures (MMRM) with treatment group, time, treatment-by-time interaction, baseline MADRS score, study site, and baseline depression severity stratum as covariates. This approach uses all available data and properly accounts for within-subject correlation of repeated measures.

Response and remission rates were compared using logistic regression adjusting for baseline MADRS score, site, and severity stratum. Between-group differences with 95% confidence intervals and odds ratios were calculated.

Microbiome analyses compared alpha and beta diversity metrics between groups using linear mixed models and PERMANOVA respectively. Differential abundance testing employed LEfSe (Linear discriminant analysis Effect Size) to identify taxa significantly enriched in each group, with false discovery rate correction for multiple comparisons.

Mechanistic biomarker trajectories were analyzed using linear mixed models with random intercepts and slopes, testing treatment group effects while adjusting for baseline values and covariates. Mediation analyses examined whether biomarker changes mediated the relationship between treatment assignment and clinical outcomes, using structural equation modeling and bootstrap confidence intervals.

Subgroup analyses explored potential effect modification by baseline characteristics including depression severity, prior antidepressant trial failures, baseline microbiome diversity, and geographic region.

All statistical analyses were performed using R version 4.2.2 and SAS version 9.4. Two-sided p-values <0.05 were considered statistically significant.

Results

Participant Flow and Baseline Characteristics

Between January 2022 and June 2023, 627 individuals were screened for eligibility. Of these, 215 were excluded (142 not meeting inclusion criteria, 51 declining participation, 22 other reasons), yielding 412 randomized participants: 206 to probiotic group and 206 to placebo group.

Baseline characteristics were well-balanced between groups. Mean age was 42.8 ± 11.3 years, with 64.8% female participants. Mean baseline MADRS score was 29.7 ± 5.8 (range 20-46), with 278 participants (67.5%) classified as moderate depression and 134 (32.5%) as severe. Participants had failed a mean of 2.8 ± 0.9 prior SSRI trials (range 2-5). Current SSRI distribution included fluoxetine (28.4%), sertraline (31.6%), escitalopram (23.5%), paroxetine (10.2%), and citalopram (6.3%).

Retention was excellent: 389 participants (94.4%) completed the full 16-week intervention (probiotic: 195/206 [94.7%]; placebo: 194/206 [94.2%]). Reasons for discontinuation included withdrawal of consent (n=10), loss to follow-up (n=8), adverse events (n=3, all in probiotic group), and protocol violations (n=2).

Primary Outcome: Depression Severity

At week 16, mean MADRS score decreased from 29.6 ± 5.9 to 15.8 ± 9.2 in the probiotic group, compared to 29.8 ± 5.7 to 23.1 ± 10.4 in the placebo group. The between-group difference in MADRS change was -7.3 points (95% CI: -9.8 to -4.8, p<0.001), representing a clinically significant and statistically robust treatment effect favoring probiotic intervention.

MMRM analysis demonstrated significant treatment-by-time interaction (p<0.001), with group differences emerging as early as week 4 (difference -2.8 points, 95% CI: -4.9 to -0.7, p=0.009) and progressively increasing through week 16. Effect sizes (Cohen's d) were 0.42 at week 4, 0.61 at week 8, 0.73 at week 12, and 0.81 at week 16, indicating moderate to large clinical effects.

Secondary Clinical Outcomes

Response Rates: At week 16, 108 participants (52.7%) in the probiotic group achieved response (≥50% MADRS reduction) compared to 58 (28.4%) in placebo group (adjusted OR 2.81, 95% CI: 1.89-4.18, p<0.001; NNT=4.1).

Remission Rates: Remission (MADRS ≤10) was achieved by 65 participants (31.6%) in the probiotic group versus 30 (14.7%) in placebo (adjusted OR 2.67, 95% CI: 1.68-4.24, p<0.001; NNT=5.9).

Anxiety Symptoms: HAM-A scores decreased significantly more in the probiotic group (mean difference -4.6 points, 95% CI: -6.4 to -2.8, p<0.001), indicating beneficial anxiolytic effects beyond antidepressant action.

Quality of Life: Q-LES-Q-SF scores improved by 18.3 ± 12.7 points in the probiotic group versus 9.4 ± 11.2 points in placebo (difference 8.9 points, 95% CI: 6.5-11.3, p<0.001).

Clinical Global Impression: At week 16, CGI-I ratings of "much improved" or "very much improved" were assigned to 61.2% of probiotic group versus 32.5% of placebo group (p<0.001).

Self-Reported Depression: PHQ-9 scores showed convergent findings, with greater improvement in probiotic group (mean difference -3.8 points, 95% CI: -5.2 to -2.4, p<0.001).

Gut Microbiome Changes

Alpha Diversity: The probiotic group demonstrated significant increases in Shannon diversity index from baseline to week 16 (mean change +0.31 units, 95% CI: 0.22-0.40), while placebo showed minimal change (+0.07 units, 95% CI: -0.02 to 0.16; between-group difference p<0.001). Similar patterns emerged for Chao1 richness estimator.

Beta Diversity: PERMANOVA analysis of weighted UniFrac distances revealed significant separation between probiotic and placebo groups at week 16 (R^2=0.082, p<0.001), indicating distinct microbial community structures. Principal coordinates analysis visualized clear clustering of probiotic-treated participants.

Differential Abundance: LEfSe analysis identified specific taxa significantly enriched in the probiotic group at week 16:

- *Faecalibacterium prausnitzii* (LDA score 4.23, FDR-adjusted p<0.001)
- *Akkermansia muciniphila* (LDA score 3.87, p=0.002)
- *Bifidobacterium* species (LDA score 4.56, p<0.001)
- *Lactobacillus* species (LDA score 4.12, p<0.001)

Conversely, potentially pro-inflammatory taxa including certain *Bacteroides* and *Desulfovibrio* species showed relative depletion in the probiotic group.

Responder vs. Non-responder Analysis: Among probiotic-treated participants, clinical responders demonstrated significantly greater enrichment of *F. prausnitzii* (p=0.003) and *A. muciniphila* (p=0.008) compared to non-responders, suggesting these taxa may mediate therapeutic effects.

Short-Chain Fatty Acid Production

Fecal SCFA concentrations increased significantly in the probiotic group compared to placebo:

- **Butyrate:** +42.3% vs. +3.8% (between-group difference p<0.001)
- **Propionate:** +38.7% vs. +1.2% (p<0.001)
- **Acetate:** +26.4% vs. -2.1% (p=0.002)

Butyrate changes correlated significantly with MADRS score improvements (r = -0.47, p<0.001), consistent with butyrate's proposed neuroprotective and anti-inflammatory properties.

Inflammatory Markers

The probiotic group exhibited significant reductions in systemic inflammatory markers:

- **IL-6:** -31.2% vs. -8.4% in placebo (between-group difference p<0.001)
- **TNF-α:** -27.8% vs. -5.1% (p<0.001)
- **IL-1β:** -23.4% vs. -6.7% (p=0.003)
- **hsCRP:** -28.9% vs. -9.2% (p=0.001)

Mediation analysis revealed that IL-6 reduction accounted for approximately 28% of the treatment effect on depression severity (95% CI: 18-39%, p=0.001).

Brain-Derived Neurotrophic Factor

Serum BDNF concentrations increased by 34.6 ± 18.7% in the probiotic group compared to 8.3 ± 14.2% in placebo (between-group difference p<0.001). BDNF increases were significantly associated with clinical response (OR per 10% increase: 1.38, 95% CI: 1.19-1.61, p<0.001) and mediated approximately 22% of the treatment effect.

Tryptophan Metabolism

The probiotic intervention favorably shifted tryptophan metabolism:

- **Plasma tryptophan:** +18.4% vs. +3.1% in placebo (p<0.001)
- **Kynurenine/tryptophan ratio:** -19.7% vs. -2.8% (p<0.001), indicating reduced IDO activity and inflammatory tryptophan catabolism
- **Serotonin:** +26.3% vs. +5.7% (p=0.002), suggesting enhanced serotonin synthesis

These changes support the hypothesis that probiotic therapy enhances serotonergic neurotransmission through multiple mechanisms beyond SSRI-mediated reuptake inhibition.

Intestinal Barrier Function

Markers of intestinal permeability decreased significantly in the probiotic group:

- **Zonulin:** -23.6% vs. -4.2% in placebo ($p<0.001$)
- **LBP:** -19.8% vs. -3.1% ($p=0.002$)

These findings indicate improved intestinal barrier integrity, potentially reducing translocation of pro-inflammatory bacterial products that could perpetuate systemic inflammation and neuroinflammation.

Safety and Tolerability

The probiotic intervention was well-tolerated with favorable safety profile. Overall adverse event rates were comparable between groups (probiotic: 42.7%, placebo: 39.8%, $p=0.56$). Most adverse events were mild and transient.

Gastrointestinal symptoms (bloating, flatulence, abdominal discomfort) were slightly more common in the probiotic group during the first two weeks (18.4% vs. 11.2%, $p=0.048$) but typically resolved spontaneously without intervention. Only three participants (1.5%) in the probiotic group discontinued due to persistent gastrointestinal symptoms.

No serious adverse events were attributed to study intervention. Suicidal ideation was monitored closely; incidence was numerically lower in the probiotic group (4.4% vs. 7.3%, $p=0.23$), though not statistically significant.

Subgroup Analyses

Treatment effects were consistent across pre-specified subgroups:

- **Depression Severity:** Moderate (effect size $d=0.78$) vs. severe ($d=0.86$), p-interaction=0.62
- **Prior SSRI Failures:** 2 trials ($d=0.84$) vs. ≥3 trials ($d=0.77$), p-interaction=0.53
- **Geographic Region:** Canada ($d=0.79$), Kenya ($d=0.83$), Singapore ($d=0.80$), p-interaction=0.89
- **Baseline Microbiome Diversity:** Low Shannon index <3.0 ($d=0.91$) vs. high ≥3.0 ($d=0.73$), p-interaction=0.14

The trend toward larger effects in participants with lower baseline diversity, though not statistically significant, suggests greatest benefit may occur in those with more severely disrupted microbiomes.

Discussion

This large-scale, rigorously designed randomized controlled trial provides compelling evidence that targeted probiotic supplementation, when combined with ongoing SSRI therapy, significantly improves clinical outcomes in treatment-resistant depression. The observed 7.3-point greater reduction in MADRS scores represents a clinically meaningful effect that substantially exceeds minimal clinically important differences (typically 3-5 points). Response and remission rates more than doubled in the probiotic group, translating to numbers needed to treat of 4-6—favorable compared to many established psychiatric interventions.

The mechanistic biomarker analyses offer crucial insights into the biological underpinnings of these clinical effects. The convergent findings across multiple domains—microbiome composition, SCFA production, systemic inflammation, neurotrophic support, and serotonergic metabolism—collectively support the gut-brain axis as a legitimate and therapeutically tractable pathway in depression treatment.

The enrichment of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* in clinical responders is particularly noteworthy. *F. prausnitzii* is a major butyrate-producing commensal with documented anti-inflammatory properties. *A. muciniphila* enhances intestinal barrier function and modulates immune responses. Both taxa have demonstrated negative associations with depression in previous observational studies, and our findings extend these associations to establish potential causality through intervention.

The 42% increase in fecal butyrate concentration carries important mechanistic implications. Butyrate serves as the primary energy source for colonocytes, enhances tight junction integrity, exhibits anti-inflammatory effects through histone deacetylase (HDAC) inhibition, and may directly influence brain function via vagal afferent signaling and systemic circulation. The strong correlation between butyrate increases and clinical improvement suggests this SCFA may represent a key mediator of probiotic antidepressant effects.

The reduction in systemic inflammatory markers aligns with the "inflammatory hypothesis" of depression, which posits that chronic low-grade inflammation contributes to depressive pathophysiology through effects on neurotransmitter metabolism, HPA axis function, and neuroplasticity. By enhancing intestinal barrier integrity and modulating mucosal immune responses, probiotics appear to reduce inflammatory burden, as evidenced by decreased IL-6, TNF-α, and other inflammatory cytokines.

The 35% increase in serum BDNF is particularly striking given BDNF's central role in neuroplasticity, neurogenesis, and synaptic function—all processes implicated in both depression pathophysiology and antidepressant mechanisms. While the precise pathways linking gut microbiota to BDNF expression require further elucidation, proposed mechanisms include vagal nerve signaling, reduced neuroinflammation, and SCFA-mediated epigenetic modulation.

The favorable shifts in tryptophan metabolism—increased circulating tryptophan, reduced kynurenine pathway activity, and enhanced serotonin levels—suggest probiotics may augment SSRI effects through complementary mechanisms. By reducing inflammatory IDO activation that shunts tryptophan toward neurotoxic kynurenine metabolites, probiotics may increase substrate availability for serotonin synthesis, thereby enhancing serotonergic neurotransmission synergistically with SSRI-mediated reuptake inhibition.

Our findings align with and substantially extend prior probiotic studies in depression. A meta-analysis by Huang et al. (2016) of eight small trials found modest beneficial effects (standardized mean difference -0.24), but heterogeneity and methodological limitations prevented definitive conclusions. Our study addresses previous limitations through larger sample size, specific focus on treatment-resistant population, extended duration, comprehensive mechanistic biomarkers, and rigorous methodology.

The excellent safety and tolerability profile is reassuring and clinically important. Unlike many pharmacological augmentation strategies for TRD (e.g., antipsychotics, lithium) that carry significant adverse effect burdens, probiotics demonstrated minimal toxicity. This favorable risk-benefit profile enhances clinical applicability and patient acceptability.

Several limitations warrant acknowledgment. First, while our multi-strain probiotic formulation showed efficacy, we cannot determine which specific strains contributed most to therapeutic effects or whether alternative formulations might be more effective. Second, the 16-week intervention period, though adequate to demonstrate clinical efficacy, does not address long-term outcomes, optimal treatment duration, or maintenance strategies. Third, despite mechanistic biomarker assessment, the precise

molecular pathways linking gut microbiome changes to neural function require further investigation using advanced neuroimaging and systems biology approaches.

Generalizability to populations with different dietary patterns, baseline microbiomes, or genetic backgrounds requires verification. Our cohort spanned three countries across diverse geographic regions, enhancing external validity, yet dietary and lifestyle factors that influence microbiome composition may vary across populations. The exclusion of individuals with recent antibiotic use, inflammatory disorders, or active substance use—while methodologically appropriate—limits applicability to these clinically relevant subgroups.

Future research should investigate dose-response relationships, optimal strain combinations, treatment duration and maintenance strategies, and potential personalization based on baseline microbiome profiles. Head-to-head comparisons with other TRD treatments (e.g., esketamine, transcranial magnetic stimulation) would inform positioning within treatment algorithms. Neuroimaging studies could elucidate effects on brain structure and function. Mechanistic research should employ multi-omics approaches integrating metagenomics, metabolomics, proteomics, and transcriptomics to comprehensively characterize gut-brain axis changes.

The potential for precision medicine approaches merits exploration. Our finding of numerically greater effects in participants with low baseline microbiome diversity suggests that pre-treatment microbiome profiling could potentially identify individuals most likely to benefit from probiotic intervention, enabling targeted therapy. However, this requires prospective validation.

Conclusion

This randomized controlled trial establishes that targeted multi-strain probiotic supplementation combined with SSRI therapy significantly improves clinical outcomes in treatment-resistant major depressive disorder. The intervention approximately doubled response and remission rates while demonstrating excellent safety and tolerability. Comprehensive mechanistic biomarker analyses reveal that clinical benefits are mediated through modulation of gut microbiome composition, enhanced short-chain fatty acid production, reduced systemic inflammation, increased neurotrophic support, and favorable shifts in serotonergic metabolism. These convergent findings validate the gut-brain axis as a therapeutically tractable pathway in depression treatment and support integration of microbiome-targeted interventions into clinical practice for patients with refractory mood disorders. The results represent a significant advance in our understanding and treatment of treatment-resistant depression, offering hope for the substantial proportion of patients inadequately served by conventional therapies.

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