

THE ROLE OF THE MICROBIOTA-GUT-BRAIN AXIS IN PSYCHIATRY AND BEYOND

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INTRODUCTION

The idea that bacteria in the human gut could play a role in mental health is not revolutionary, and the prehistory of this area provides important context for present-day research. The concept of auto-intoxication, a state of being poisoned by toxic substances produced within the body, was widely accepted in the 1800's and ultimately led to methods for dealing with 'cases requiring change of the intestinal flora', such as those used to treat illnesses that would be characterized as mirroring today's mental illnesses by people such as Dr. John Kellogg at the Battle Creek Sanitarium (*Kellogg*, 1919). The microbiota-gut-brain (MGB) axis is a bidirectional pathway between the brain and microbiota in the gastrointestinal system (*McVey Neufeld et al.*, 2024). It is comprised of humoral, neural, and cellular pathways that ultimately connect the gut microbiota with the central nervous system

(CNS). That the gut microbiota can impact the symptoms of mental illness through a variety of purported mechanisms has been extensively researched and work has been done to evaluate this connection as a potential therapeutic target via faecal microbiota transplantation (FMT) or probiotics (*Wallace et al.*, 2020; *Settani et al.*, 2021; *Chung et al.*, 2022; *Queiroz et al.*, 2022; *Nikolova et al.*, 2023). This has resulted in the intriguing possibility of the MGB axis being a possible treatment target. The probiotic literature for mental illness is mixed (*Hofmeister et al.*, 2021), however, in part because probiotics are not designed to change the gut microbiome (*Wallace et al.*, 2017). FMT may offer a better biotherapeutic option as evidence suggest it may result in more long-term effects on the MGB axis (*Wallace et al.*, 2017; *Weingarden et al.*, 2017; *Chinna Meyyappan et al.*, 2020).

INFLUENCE OF ANTI-DEPRESSANT MEDICATIONS ON THE GUT MICROBIOTA OF PATIENTS WITH DEPRESSION

This led us to explore translational clinical trials using FMT but prior to this, we had done research to show that anti-depressant medications alter the gut microbiota of patients with depression, indicating support for studies taking this further. As published earlier (*Bharwani et al.*, 2020), we recruited participants were between 18 and 60 years of age with Major Depressive Disorder (MDD)

as confirmed by the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (*American Psychiatric Association*, 1994) and moderate depression as indicated by a Montgomery-Asberg Depression Rating Scale (MADRS; *Montgomery and Asberg*, 1979) score of >19. Exclusion criteria included psychiatric medication use (other than sedatives for sleep and benzodiazepines) at

minimum 1 week prior to visit 1, medical conditions or current use of medications or natural health products known to impact the microbiota, and current substance abuse. After the screening visit, participants were given a 3-day food diary that was returned prior to the baseline visit and repeated at 3- and 6-month assessments. Anthropomorphic measurements (height, weight, and waist circumference) were also completed, and the MADRS administered, at the same time points. Participants then initiated therapy with Citalopram or Escitalopram. Specimens were stored at 80°C. Bacterial community profiling using 16S rRNA gene sequencing was carried out using a modified bar-coded Illumina sequencing method (Chung et al, 2022). Using data rarefied in QIIME at the lowest sequencing depth, phylogenetic diversity was analysed. For beta diversity, Jackknife resampling was used to generate Bray-Curtis distances. To identify differentially abundant operational taxonomic units (OTUs), OTUs not observed in 75% of samples were eliminated, and the data set was analysed using the DESeq2 software package and the Benjamini-Hochberg procedure.

In total, fifteen participants (mean age = 36.9; SD = 12.9; 12 women) were studied. The average baseline MADRS score was 22.53 (SD = 6.63). At 6 months, 11 participants were classified as remitters (MADRS < 12) and 4 as non-remitters (MADRS > 13). Citalopram was taken by 3 participants in the remitter group and 1 participant in the non-remitter group; the remaining 11 took Escitalopram. Remitters exhibited greater diversity at baseline than non-remitters (Mann-Whitney U test = 5, $P = 0.026$). Bray-Curtis distances revealed no differences in variability (Mann-Whitney U test = 138, $P = 0.517$) and a lack of community clustering based upon eventual treatment response

(PERMANOVA; $F = 0.720$, $P = 0.824$). At baseline, 22 OTUs were differentially abundant between the remitter and non-remitter groups.

Comparisons at 3- and 6-months following treatment showed no change in diversity in remitter or non-remitters. Differences in diversity between groups were no longer significant at 3 months but were again significant at 6 months ($F = 4.773$, $P = 0.048$, post hoc $P < 0.05$). Analysis of the composition in remitters showed no clustering by time point (PERMANOVA; $F = 0.262$, $P = *1$) and no changes in within-subject distances (Friedman statistic = 0.546, $P = 0.844$). Similarly, non-remitters showed no clustering of the community across treatment (PERMANOVA; $F = 0.433$, $P = 0.951$) or changes in within-subject distances (Friedman statistic = 3.50, $P = 0.273$).

Next, we examined whether specific OTUs were altered after treatment. In remitters, a single OTU was altered (a *Clostridiales*, elevated at 6 months). No OTUs were altered in non-remitters. At 3 months, 35 OTUs were significantly different between remitters and non-remitters but 16 were no longer different by the 6-month time point. At 6 months, 42 OTUs were different between groups: 23 were unique while the remaining 19 were also differentially abundant at 3 months. There was no impact of dietary composition as analysed by Nutritionist Pro diet analysis software or antidepressant exposure.

Our findings indicated that antidepressant treatment affects the microbiota of MDD patients at the OTU level, based on response to treatment. Assessment at 3 and 6 months following treatment revealed no changes to the diversity or profile of the community relative to baseline, which is consistent with previous work demonstrating the lack of effects of antidepressant medications on bacterial taxa (Queiroz et al., 2022).

It was therefore interesting that notable differences in the microbial community were observed in analyses based on treatment response. Despite similar baseline depression severity scores, non-remitters exhibited lower diversity prior to treatment initiation. Additionally, 22 microbial groups were differentially abundant between response groups. Our data provide compelling preliminary evidence that baseline differences in the microbiota may be correlated with antidepressant medication efficacy.

To further examine the impact of antidepressant medications, we compared changes in the microbiota in remitters and non-remitters across 6 months of treatment. There was no change in diversity or overall profile in either response group, paralleling our findings of limited community-wide changes. However, baseline differences between remitters and non-remitters

were still evident after 6 months of treatment: Remitters continued to exhibit greater diversity. These observations further suggest a microbial community signature distinguishing treatment response groups.

Our data indicate that antidepressant medications may alter the gut microbiota of patients with MDD and outline the disparate nature of their effects in remitters versus non-remitters. Limitations include the small sample size and the lack of a placebo controls. Larger studies are needed to investigate whether the gut microbiome may serve as a potential biomarker to aid in guiding treatment. But this work supported the concept of a microbiota phenotype associate with treatment response in depression (*Bharwani et al., 2020*), and added to the growing support for the gut microbiome as a target for the treatment of mental illness. This led us in to trials.

CLINICAL TRIALS USING FAECAL MICROBIOTA TRANSPLANTATION

A growing body of literature has found that individuals with Bipolar Disorder (BD) have alterations in the gut microbiome composition or function and that these alterations are associated with illness severity in BD (*Coello et al., 2019; Painold et al., 2019*). We decided to investigate this with a phase 2/3, double-blind randomized control trial (RCT). In this study, all participants with BD on, but failing to respond to, first-line treatment were given a medical grade bowel preparation and then randomized to receive either (1) allogenic FMT from healthy donors or (2) autologous FMT with their own faeces by colonoscopy. FMT was manufactured into a liquid filtrate and administered via colonoscopy by a trained gastroenterologist. Participants were subsequently

followed for 24 weeks for assessment of primary and secondary study outcomes.

In terms of data, we recruited adults 18 to 65 years of age, who had a Mini-International Neuropsychiatric Interview (*Sheenan et al., 1998*) confirmed diagnosis of BD (type I or II) on a stable first-line treatment (*Yatham et al., 2018*) or ≥ 8 weeks prior to study entry, who were currently experiencing a depressive episode. A participant met inclusion criteria if their current episode was characterized by a MADRS (*Montgomery and Asberg, 1979*) score at screening and baseline of ≥ 12 . Exclusion criteria included a Young Mania Rating Scale (*Young et al., 1978*) score of ≥ 12 at screening; Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria (*American Psychiatric*

Association, 1994) for substance abuse within the past 6 months or lifetime dependency; active suicidal ideation, an active eating disorder; schizophrenia or schizoaffective disorder; chronic gastrointestinal diseases; conditions causing immunosuppression; a significant bleeding disorder; a history of incomplete colonoscopy; pregnancy, regular use of non-steroidal anti-inflammatory drugs, antibiotics, prebiotics or probiotics for medical purposes, or iron supplements within 3 months prior to study entry.

Participants were randomly allocated (1:1) to groups receiving either allogenic or autologous FMT and once enrolled, FMT administration was delivered via colonoscopy after a standard bowel cleanse consisting of 4 L of an over-the-counter polyethylene glycol laxative solution. The primary outcome of this study is to determine the effectiveness, safety, and tolerability of FMT as an adjunctive treatment in adults with BD depression, as well as the viability of a larger trial. Feasibility was measured by participant recruitment, retention and completion rates, adherence to the intended study protocol, participant acceptability, and robustness of study methodology. Safety was measured by the assessment of severe adverse events while Tolerability was monitored by the Toronto Side Effect Scale (TSES) (Vanderkooy et al., 2002), with each symptom rated for both frequency and severity on a five-point scale (1 = Never/No trouble; 5 = Everyday/Extreme trouble), as well as weight changes. Adverse events were also assessed via a symptom diary reviewed at each visit. Effectiveness, the primary clinical outcome, was measured by change in MADRS score from baseline to the end of follow-up.

At the end of the study, MADRS scores significantly improved from baseline to the last visit in both treat-

ment arms. There was no significant difference between allogenic FMT (16.74-point improvement) and autologous FMT (15.4-point improvement) regarding clinical efficacy ($t = -0.47$, p -value = .64, 95% confidence interval [CI] = -7.3 - 4.6). Microbiota analysis showed that allogenic FMT lead to a bacterial profile similar to the healthy donor and increased bacterial diversity at the 6-month mark, whereas those receiving autologous FMT did not. The intervention was well tolerated with no significant adverse events. Recruitment, randomization, and retention metrics support feasibility of a larger trial.

There were clear limitations associated with this FMT methodology, related to the many unknowns still existing in this field, including the use of a single dose FMT, as there is still debate regarding the best frequency of FMT to produce appropriate results. Colonoscopy (Cammarota et al., 2017) was chosen as the method of intervention as it is reasonable to believe that better FMT distribution increases the chance of donor microbiota engraftment, which has been shown to be associated with treatment outcome (Wilson et al., 2019) and current evidence indicates this is better achieved with colonoscopy (Skjevling et al., 2023). A recent study of FMT by enema for depression, however, indicates other methods of administration may be adequate as well (Green et al., 2023). No antibiotic was used as evidence indicated that it could impact engraftment (Zhao et al., 2020; Singh et al., 2022), but again, the data is equivocal and thus further work needs to be done (Pocari et al., 2023). The use of bowel preparation may also have an impact on outcomes (McGuinness et al., 2024) but evidence from studies showing efficacy after the 6 months indicates that the effect may be transient (Brugeman et al., 2024). We also notice trends toward increased diversity in the

allogenic group, indicating something other than the bowel preparation was having an effect.

This study was designed as a feasibility study to inform outcomes of future research, as such studies are essential to inform key next steps. It adds to the pos-

itive findings of two case reports reporting the efficacy of FMT in refractory BD (*Hinton, 2020*) and a probiotic RCT showing improvements in mania (*Parker et al., 2022*) building on the evidence base supporting microbial manipulation being a viable therapeutic target

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