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Study protocol

Keywords: Fecal microbiota transplantation, Schizophrenia, Microbiota-gut-brain axis, Randomized controlled trial, Adjunctive therapy, Clinical trial protocol

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Fecal Microbiota Transplantation as an Adjunctive Therapy for Schizophrenia: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial Protocol

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Abstract

Background: Schizophrenia is a chronic, severe mental disorder with a substantial

global disease burden. Current antipsychotic medications demonstrate limited efficacy on negative symptoms and cognitive impairment and are frequently associated with significant gastrointestinal adverse effects. Emerging evidence implicates gut microbiota dysbiosis in the pathophysiology of schizophrenia via the microbiota-gut-brain axis, suggesting that fecal microbiota transplantation (FMT) may offer a novel adjunctive therapeutic strategy. This trial aims to evaluate the efficacy and safety of FMT as an adjunctive therapy in patients with non-treatment-resistant schizophrenia.

Methods: This is a multicenter, randomized, double-blind, placebo-controlled trial. A total of 80 patients with schizophrenia, aged 18–65 years, with a disease duration ≤ 10 years and a Positive and Negative Syndrome Scale (PANSS) total score ≥ 60, will be enrolled from 11 centers across China and randomized in a 1:1 ratio to either the FMT group (receiving standard antipsychotic treatment in combination with FMT capsules) or the placebo group (receiving standard antipsychotic treatment in combination with placebo capsules). The FMT capsules are prepared from healthy donor feces, with each capsule containing 1.6×10^{11} colony-forming units of active microbiota. The intervention regimen consists of 10 capsules daily for 6 days, followed by 5 capsules daily for 12 days, totaling 120 capsules over 18 days. The primary outcome is the change in PANSS total score during the 24-week follow-up. Secondary outcomes include cognitive function assessed by the MATRICS Consensus Cognitive Battery (MCCB), safety assessments using the Treatment Emergent Symptom Scale (TESS), Clinical Global Impression (CGI), Gastrointestinal Symptom Rating Scale (GSRS),

Bristol Stool Form Scale (BSFS), and adverse event/serious adverse event reporting, as well as laboratory parameters (complete blood count, liver and renal function, lipid profile, fasting insulin) and gut microbiota diversity. Assessments are scheduled at baseline, day7, day 18, week 4, week 8, week 12, and week 24.

Discussion: This trial will provide high-level evidence on the efficacy and safety of FMT as an adjunctive therapy for non-treatment-resistant schizophrenia and may elucidate mechanistic pathways involving the microbiota-immune-cognition axis. Regardless of the direction of results, the findings will inform the design of future studies and contribute to the evidence base for microbiome-based interventions in psychiatric disorders.

Trial Registration: *ClinicalTrials.gov* (NCT05850585). Registered on 26 May 2023.

Keywords: Fecal microbiota transplantation; Schizophrenia; Microbiota-gut-brain axis; Randomized controlled trial; Adjunctive therapy; Clinical trial protocol

Contributions to the literature

- FMT has been proposed as a potential adjunctive therapy for schizophrenia, but completed evidence lags behind registered trial protocols. Existing protocols focus on treatment-resistant cases; this trial targets the much larger non-treatment-resistant population, a group not yet addressed in any published RCT protocol.

- This multicenter RCT is the first to evaluate FMT specifically for non-treatment-resistant schizophrenia, using a rigorous design with comprehensive clinical and biological measurements.
- If completed, this trial will provide the definitive evidence to establish whether FMT has a role in schizophrenia treatment. Regardless of outcome, it will transform the field by moving FMT from registered protocols to actionable answers for clinicians and patients.

1. Introduction

Schizophrenia is a chronic, severe, and lifelong disabling mental disorder that typically emerges in late adolescence or early adulthood, characterized by significant abnormalities in cognition, thought, emotion, and volition. According to the Global Burden of Disease Study 2021, approximately 23.18 million people worldwide were affected by schizophrenia in 2021, with a global point prevalence of approximately 0.32%(1, 2). Schizophrenia accounted for an estimated 14.82 million disability-adjusted life years (DALYs), ranking among the top 20 causes of disability worldwide. Alarmingly, a systematic review and meta-analysis found that only approximately 1 in 7 (about 14%) of treated patients achieve recovery defined by improvements in both clinical and social domains, and this proportion has not increased despite major advances in treatment options over recent decades(3). Schizophrenia has thus become a globally recognized public health priority.

Current first-line antipsychotic medications, including atypical antipsychotics such

as olanzapine, quetiapine, risperidone, aripiprazole, amisulpride, and paliperidone, demonstrate efficacy in alleviating positive symptoms but show limited improvement in negative symptoms and cognitive impairment. Meta-analyses have confirmed that while atypical antipsychotics offer advantages in overall symptom improvement, they show limited and time-dependent efficacy on negative symptoms and cognitive impairment(4). Regarding side effects, atypical antipsychotics are commonly associated with anticholinergic adverse effects including dry mouth, constipation, blurred vision, and urinary retention(5). Constipation, in particular, has a prevalence rate of 28.1% to 36.3% among antipsychotic-treated patients(6). It not only compromises medication adherence but may also progress to serious complications such as paralytic ileus, bowel ischemia, intestinal perforation, and even life-threatening conditions if left untreated(7). Therefore, there is an urgent need to identify adjunctive therapeutic strategies that can enhance antipsychotic efficacy while alleviating gastrointestinal adverse effects.

In recent years, the role of gut microbiota in modulating brain function and behavior through the “microbiota-gut-brain” axis has garnered increasing attention(8, 9). Accumulating evidence implicates gut microbiota dysbiosis in the pathophysiology of schizophrenia(10). Our previous research demonstrated that gut microbiota from patients with schizophrenia can modulate the tryptophan-kynurenine metabolic pathway in mice and induce schizophrenia-related behaviors(11). Furthermore, 16S rRNA high-throughput sequencing analyses have revealed significant alterations in gut microbiota composition in patients with schizophrenia(12), suggesting that gut

111 microbial features may serve as auxiliary biomarkers for clinical diagnosis.

112 Fecal microbiota transplantation involves the transfer of functional microbiota from
113 rigorously screened healthy donors into the patient's gastrointestinal tract via specific
114 medical techniques (e.g., oral capsules, enema, endoscopy), with the aim of
115 reconstructing a healthy intestinal microecology. Compared to probiotics, FMT offers
116 higher species diversity, including bacteria, viruses, fungi, and their metabolites, and
117 can re-establish the intestinal barrier and regulate immunity through the
118 transplantation of a complete donor-derived ecosystem(13). FMT has already been
119 recommended for recurrent *Clostridium difficile* infection and has demonstrated
120 positive effects in improving both gastrointestinal and behavioral symptoms in
121 patients with autism spectrum disorder(14).

122 A systematic search of *ClinicalTrials.gov* (search date: July 28, 2026; filters:
123 condition “Schizophrenia”, study type “Interventional”) yielded 3,547 registered
124 studies, while a parallel search of the Chinese Clinical Trial Registry (ChiCTR)
125 yielded 308 studies. Despite this substantial pool, those specifically investigating
126 FMT in schizophrenia constitute only five discrete investigator-initiated trials (IITs) to
127 date (Error! Unknown switch argument.).

128

129 **Table 1** The currently identifiable FMT IITs in schizophrenia

Trial Identifier	Title	Population	Design	Sample Size	Status
NCT05850585	A Study of the	Non-tr	Double-bli	80	Recruiting

	Efficacy of Fecal Microbiota Transplantation (FMT) in the Treatment of Schizophrenia	eatmen t-resist ant schizo phrenia , duratio n ≤10 years, PANS S ≥60	nd, Randomiz ed Controlled Trial, oral FMT capsules-p lacebo		
NCT04001439	Fecal Microbiota Transplantation for Major Depression in Schizophrenia	Schizo phrenia with comor bid treatme nt-resis tant depress ion	Single Group Assignme nt, Open-labe l, oral capsules	3088	Unknown status
ChiCTR24000 92799	Efficacy and safety of Fecal Microbiota Transplantation (FMT) by gastroscopy in the treatment of patients	Treatm ent-resi stant schizo phrenia (TRS)	Double-bli nd, three-arm: gastroscop y FMT vs oral	120	Protocol published

	with refractory schizophrenia: a randomized, double-blind, placebo-controlled trial		capsule FMT vs placebo		
ChiCTR2100045243	A Randomized Clinical Trial of Fecal Microbiota Transplant for Treatment-Resistant Schizophrenia	Treatment-resistant schizophrenia (TRS)	Blinded, Randomized Controlled Trial, parallel	40	Recruiting
ChiCTR2600121876	Clinical Efficacy and Mechanism of Fecal Microbiota Transplantation in the Treatment of Psychotropic Drug-Related Constipation	Psychotropic Drug-Related Constipation	Random parallel control, blind	100	Not yet recruiting

This scarcity underscores the nascent stage of clinical translation for microbiome-based interventions in schizophrenia, with the majority of published work remaining at the preclinical stage. Among these five IITs, only one full trial protocol, the TRS-FMT protocol (ChiCTR2400092799), has been published in a

peer-reviewed journal, and it specifically targets treatment-resistant schizophrenia (TRS)(15). However, to date, no published randomized controlled trial protocol has specifically addressed the substantially larger population of non-treatment-resistant schizophrenia patients(16). The present study is therefore positioned as the only publicly available, peer-reviewed protocol targeting this broader group, offering potential advantages for earlier intervention before treatment resistance consolidates.

While the TRS-FMT protocol provides valuable insights into a difficult-to-treat subgroup, the present study distinguishes itself through its focus on non-TRS patients, a two-arm design optimized for statistical power on the primary endpoint, exclusive use of oral capsules for scalability, systematic cognitive assessment via MCCB, comprehensive metabolic monitoring, and a 24-week follow-up to assess durability (Error! Unknown switch argument.).

Table 2 Comparative Analysis: NCT05850585 vs. Published TRS-FMT Protocol (ChiCTR2400092799)

Dimension	NCT05850585 (Present Study)	TRS-FMT Protocol (ChiCTR2400092799)
Population	Non-treatment-resistant schizophrenia	Treatment-resistant schizophrenia (TRS)
Disease duration	≤ 10 years	≤ 15 years
Rationale	Broader patient population with greater generalizability	Higher unmet need, but narrower applicability
Design	Two-arm (FMT vs placebo)	Three-arm: gastroscopy FMT vs oral capsule FMT vs double-placebo control (1:1:1 ratio)
Sample size	80 (40 per arm)	120 (40 per arm)
Route	Oral capsules only	Gastroscopy (intraduodenal) vs oral capsules
Dose	1.6×10^{11} CFU/capsule, 10 capsules/day × 6 days, then 5 capsules/day × 12 days	Not specified in available information
Standardization	Single standardized oral regimen	Exploratory comparison of two routes
Blinding	Double-blind (capsules)	Double-blind (double-placebo design: gastroscopy + capsules)
Multi-omics	Metagenomics, metatranscriptomics, metabolomics, immunomics	Microbiomics and metabolomics

Primary outcome	PANSS total score change at 24 weeks	PANSS total score change from baseline to 12 weeks
Assessment points	7 (baseline, day7, day18, week4, week8, week12, week24)	4 (baseline, week4, week8, week12)
The longest follow-up period	24 weeks	12 weeks

Note: CFU, Colony-Forming Units.

Against this background, we designed this multicenter, randomized, double-blind, placebo-controlled trial to investigate whether FMT as an adjunctive therapy can synergistically improve psychopathological symptoms, restore gut microbial homeostasis, modulate immune function, and mitigate antipsychotic-induced metabolic disturbances in patients with non-treatment-resistant schizophrenia receiving conventional antipsychotic treatment.

2. Objectives

2.1 Primary Objective

To evaluate the efficacy of FMT as an adjunctive therapy on psychopathological symptoms in patients with schizophrenia, as measured by the change in PANSS total score, and to comprehensively assess its safety profile through systematic adverse event monitoring and laboratory evaluations.

2.2 Secondary Objectives

(1) To investigate whether FMT adjunctive therapy restores gut microbial ecosystem homeostasis, as assessed by metagenomic species/strain composition, metatranscriptomic functional activity, and metabolomic profiles (SCFAs, bile acids, tryptophan metabolites);

(2) To explore whether FMT-induced microbial remodeling is associated with systemic immune rebalancing, evaluated through high-dimensional immunophenotyping (spectral flow cytometry), inflammatory proteomics, and PBMC functional assays;

(3) To evaluate whether FMT adjunctive therapy mitigates antipsychotic-induced glucose-lipid metabolic disturbances, as determined by longitudinal changes in fasting glucose, fasting insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and full lipid profiles.

3. Methods and Design

3.1 Study Design and center

This multicenter, randomized, double-blind, placebo-controlled trial aims to evaluate the efficacy and safety of FMT as an adjunctive therapy in patients with non-treatment-resistant schizophrenia. The lead center is the First Affiliated Hospital of Xi'an Jiaotong University. Participating centers include: The First Affiliated Hospital of Zhengzhou University, West China Hospital of Sichuan University, The Second Xiangya Hospital of Central South University, Suzhou Guangji Hospital, Guangzhou Brain Hospital, Ankang Hanbin District Third Hospital, Ankang Central

Hospital, Xunyang Disabled Persons'Federation Bo'ai Hospital, Xianyang Central Hospital, Ankang Jinzhou Mental Health Rehabilitation Hospital, and Ankang Antai Hospital. The overall study design, including participant allocation, intervention, and assessment schedule, is summarized in **Figure 1**.

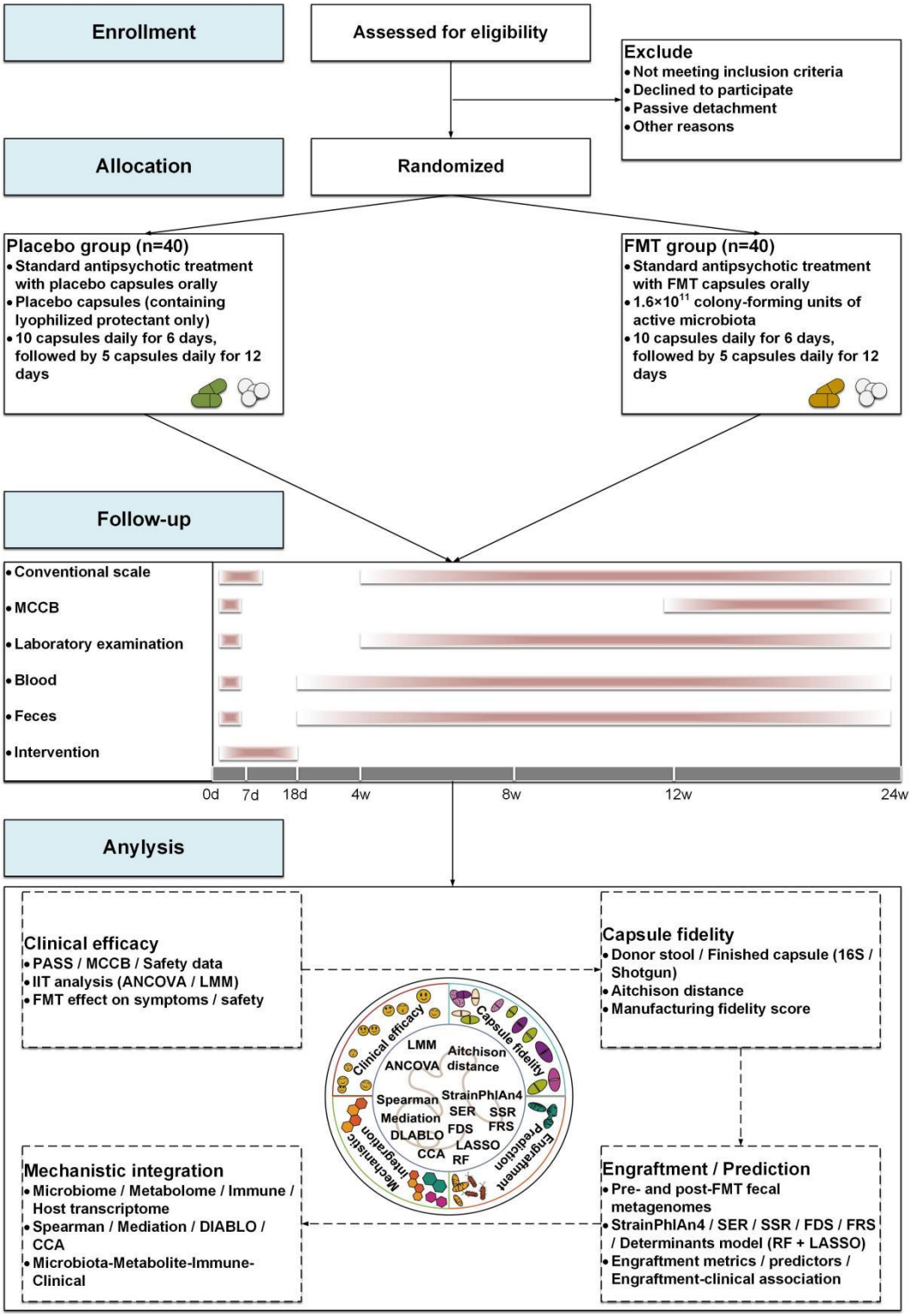


Figure 1. CONSORT flow diagram of the trial design.

Eligible patients with non-treatment-resistant schizophrenia (PANSS total score ≥ 60, disease duration ≤ 10 years) will be randomized 1:1 to receive either FMT capsules or placebo capsules (identical in appearance, weight, and odor, containing lyophilized protectant only)

adjunctive to standard antipsychotic treatment, with both groups following the same administration schedule. Follow-up assessments are scheduled at baseline (V1), day 7 (V2), day 18 (V3, end of intervention), week 4 (V4), week 8 (V5), week 12 (V6), and week 24 (V7, endpoint). Assessments include clinical scales (PANSS, MCCB, TESS, CGI, GSRS, BSFS), laboratory examinations (blood counts, liver/renal function, lipid profile, fasting insulin), and biospecimen collection (blood and feces) for multi-omics profiling. The analytical framework comprises four layers: Layer 1 evaluates the clinical efficacy of FMT versus placebo (primary and secondary outcomes). Layer 2 assesses capsule fidelity by comparing the finished capsule microbiota to the original donor stool. Layer 3 quantifies donor strain engraftment, identifies its determinants, and examines its association with clinical outcomes. Layer 4 integrates multi-omics data (microbiome, metabolome, immune, and host transcriptome) to explore mechanistic pathways. Clinical scale abbreviations: FMT, fecal microbiota transplantation; PANSS, Positive and Negative Syndrome Scale; MCCB, MATRICS Consensus Cognitive Battery; TESS, Treatment Emergent Symptom Scale; CGI, Clinical Global Impression; GSRS, Gastrointestinal Symptom Rating Scale; BSFS, Bristol Stool Form Scale. Statistical and analytical abbreviations are defined in the main text abbreviations table.

3.2 Sample Size

A total of 80 participants will be enrolled, with 40 in the FMT group and 40 in the placebo group. The sample size was determined based on an estimated effect size for PANSS score reduction derived from preliminary studies, with a power of 80% and a two-sided alpha of 0.05, accounting for a 15% dropout rate.

220 ### 3.3 Participants

221 Eligibility criteria, including inclusion, exclusion, and withdrawal criteria, are
222 summarized in Error! Unknown switch argument..

223

224 **Table 3** Eligibility Criteria

Category	Criteria
Inclusion Criteria	➢ Age 18–65 years, both sexes; ➢ DSM-5 diagnosis of schizophrenia, disease duration ≤ 10 years; ➢ PANSS total score ≥ 60 (at least 3 positive or 3 negative items ≥ 3); ➢ Junior high school education or above; ➢ Signed informed consent
Exclusion Criteria	➢ Pregnancy or lactation; ➢ Severe somatic diseases (hepatic/renal failure, cancer, active infection, inflammatory bowel disease); ➢ Refractory infections or immunocompromised status; antibiotic or immunosuppressant use within the past month; ➢ Other neuropsychiatric disorders; ➢ Fixed alcohol consumption; ➢ BMI outside 18.5–28.0
Withdrawal Criteria	➢ Severe adverse events; ➢ Symptom deterioration requiring antipsychotic class change; ➢ Voluntary withdrawal; ➢ Concurrent severe infection or use of anti-inflammatory drugs during follow-up;

- Poor adherence (< 80 capsules consumed)

All participating centers are tertiary or specialized psychiatric hospitals with established departments of psychiatry and experience in conducting clinical trials in schizophrenia. Each site has access to appropriate facilities for participant recruitment, clinical assessment, laboratory sample processing, and long-term follow-up. Site investigators are licensed psychiatrists with a minimum of 5 years of clinical experience in schizophrenia treatment and management. All clinical assessors responsible for administering PANSS, MCCB, CGI, TESS, GSRS, and BSFS are required to complete centralized training and achieve an intraclass correlation coefficient (ICC) $\geqslant$ 0.85 for PANSS total score and MCCB domain scores prior to study initiation. FMT capsules are prepared by a single third-party manufacturer (Xi'an Shanjun Biotechnology Co., Ltd.) and delivered to all sites in a standardized manner. No site-specific variation in intervention preparation or administration is permitted.

Baseline demographic and clinical characteristics will be collected for all enrolled participants prior to randomization, including: age, sex, ethnicity, years of education, marital status, employment status, living arrangements, socioeconomic status, height, weight, body mass index (BMI), age at first onset of schizophrenia, total disease duration, number of hospitalizations, family history of psychiatric disorders, comorbid physical illnesses, substance use history (tobacco, alcohol), and medication history (current and previous antipsychotic regimens, doses, and treatment adherence).

Baseline clinical assessments will include PANSS total and subscale scores, MCCB cognitive domain scores, CGI severity, TESS, GSRS, and BSFS. Laboratory parameters (CBC, liver and renal function tests, lipid profile, fasting glucose, fasting insulin, hs-CRP) and 12-lead ECG will also be recorded at baseline. Baseline characteristics will be summarized by treatment group using descriptive statistics (means with standard deviations for continuous variables; frequencies and percentages for categorical variables) to ensure comparability between groups.

Recruitment is anticipated to commence in November 2025 and continue for approximately 18 months, with a target enrollment of 80 participants. The final follow-up visit (Week 24) for the last enrolled participant is expected to be completed by February 2027. Recruitment periods and follow-up dates will be recorded for each participant and reported in the final study results.

3.4 Randomization, Blinding and Unblinding

The allocation sequence will be generated by an independent biostatistician not involved in participant enrollment or outcome assessment, using a block randomization method (block size of 4) stratified by center with a computer-generated algorithm (SAS PROC PLAN). Participant enrollment and group assignment will be performed by site investigators who have no access to the allocation sequence. Once a participant is deemed eligible and provides written informed consent, the site investigator will contact the central randomization center via the Interactive Web Response System (IWRS) to obtain the next sequential treatment allocation.

Participants, outcome assessors, and data analysts will remain blinded throughout

the study. FMT and placebo capsules are identical in appearance, weight, and odor, and will be uniformly prepared and coded by a third-party company (Xi'an Shanjun Biotechnology Co., Ltd.). Capsules will be administered orally by trained study nurses at each participating center; participants will be instructed to take capsules with water in the morning under observation for the first dose of each day. Adherence will be calculated as the number of capsules consumed divided by the number prescribed (≥80% defined as adherent; <80% triggers withdrawal). To ensure intervention fidelity, batch-to-batch consistency will be verified by 16S rRNA sequencing and viable counting, with formal fidelity assessment detailed in Section 4.2 (capsule–stool Aitchison distance). The integrity of blinding will be assessed at the final visit using Bang's Blinding Index, and any deviations from the intended intervention will be recorded with reasons.

Consistent with ICH E6 (R2) Section 5.13.4 and CONSORT 2025 Item 20, a two-stage unblinding procedure will be implemented. The first unblinding (after database lock and blind data review) will reveal only group labels (e.g., Group A vs. Group B) without identifying active treatment versus placebo, allowing the statistician to perform the primary analysis while remaining blinded to treatment identity. The second unblinding (after the statistical analysis plan is executed and the primary analysis is completed) will fully identify the active treatment group. Emergency unblinding may be performed by the Investigator of Record (IoR) through the IWRS in the event of a medical emergency where knowledge of treatment assignment is necessary for clinical management, with documentation requirements (date, time,

reason, and procedures) recorded in the eCRF; once emergency unblinding is performed, the participant will be treated as a dropout. No interim analyses are planned unless required by the Data Monitoring Committee for safety review.

3.5 Interventions

3.5.1 Group Assignments

Participants receive standard antipsychotic treatment in combination with either FMT capsules or visually identical placebo capsules. Detailed intervention protocols are summarized in **Table 4**.

Table 4 Intervention Protocol Comparison

Item	FMT Group (n = 40)	Placebo Group (n = 40)
Antipsychotic treatment	Standard antipsychotic treatment	Standard antipsychotic treatment (same as FMT group)
Capsule content	Active microbiota (1.6×10^{11} CFU/capsule)	Lyophilized protectant only (no active microbiota)
Appearance/Weight/Odor	—	Identical to FMT capsules
Dosing regimen	10 capsules/day × 6 days, then 5 capsules/day × 12 days	Same as FMT group
Total capsules	120 capsules over 18 days	120 capsules over 18 days

3.5.2 Bowel Preparation

To enhance colonization efficiency, the following bowel preparation protocol will be performed before the first transplantation: (1) Oral administration of 2L polyethylene glycol 24 hours prior to transplantation for bowel cleansing; (2) Fasting for 4 hours before and 1 hour after transplantation; (3) Morning fasting transplantation, with food and antipsychotic medication permitted 1 hour after transplantation; (4) Antibiotics and immunosuppressants are prohibited during the intervention and follow-up periods.

3.5.3 Concomitant Medication Regulations

During the treatment period, only second-generation antipsychotics (olanzapine, quetiapine, risperidone, paliperidone, aripiprazole, amisulpride, ziprasidone) are permitted as the standard antipsychotic backbone, but clozapine is prohibited. Antipsychotic doses should be titrated to therapeutic levels within 2 weeks of enrollment. Thereafter, the drug class should not be changed, though dose adjustments are allowed. Combination of two antipsychotics is permitted. Benzodiazepines, eszopiclone, zolpidem, and medications for managing side effects (trihexyphenidyl, propranolol, metoprolol) and non-probiotic laxatives are allowed. Transcranial magnetic stimulation and biofeedback therapy are permitted. Antidepressants, mood stabilizers, medications affecting body weight or glucose-lipid metabolism, and modified electroconvulsive therapy are prohibited. Any use of prohibited medications will be recorded as a protocol deviation.

All concomitant treatments, including prescription and over-the-counter

medications, non-pharmacological therapies (e.g., psychotherapy, physical therapy), and dietary supplements, will be systematically documented at each visit (V1–V7) using a standardized case report form. For each concomitant treatment, start and end dates, dosage, frequency, and indication will be recorded. Concomitant care will be summarized by treatment group in the final analysis to assess whether any between-group differences could have confounded the primary or secondary outcomes.

3.6 Outcome Measures and Assessment Schedule

A total of 7 assessment time points are scheduled: baseline (V1), day 7 (V2), day 18 (V3), week 4 (V4), week 8 (V5), week 12 (V6), and week 24 (V7).

3.6.1 Primary Outcome

PANSS total score change: Assessed at baseline, week 4, week 8, week 12, and week 24.

3.6.2 Secondary Outcomes

Cognitive function: MATRICS Consensus Cognitive Battery (MCCB) at baseline, week 12, and week 24.

3.6.3 Safety assessments

Safety will be assessed using the Treatment Emergent Symptom Scale (TESS) for adverse treatment effects, the Clinical Global Impression (CGI) for overall illness severity and improvement, the Gastrointestinal Symptom Rating Scale (GSRS) for gastrointestinal symptoms, and the Bristol Stool Form Scale (BSFS) for stool

consistency. Adverse Event and Serious Adverse Event (AE/SAE) reporting will be conducted at all visits using Common Terminology Criteria for Adverse Events (CTCAE) criteria.

3.6.4 Adverse Event Monitoring and Management

Patients are instructed to monitor for common transient adverse events, including nausea, bloating, and mild abdominal cramping, which typically resolve within 24 hours. If symptoms persist beyond 24 hours or if severe adverse events occur (e.g., fever, severe diarrhea, signs of infection), patients are advised to contact the study site immediately. The following events should be systematically recorded in the case report form (CRF) during the first month post-FMT: infectious events (fever, bacteremia, pneumonia), psychiatric symptom exacerbation (PANSS increase $\geqslant$20% from baseline), and severe gastrointestinal adverse events (intestinal obstruction, perforation, severe diarrhea). Patients should maintain a bowel movement diary for at least eight weeks post-FMT to monitor intestinal transit and treatment response

3.6.5 Laboratory parameters

Complete blood count, liver and renal function tests, lipid profile, fasting insulin, and 12-lead electrocardiogram at baseline, week 4, week 8, week 12, and week 24. The complete assessment schedule, including all outcome measures and biospecimen collection across the seven time points, is summarized in **Table 5**.

Table 5 Assessment Schedule and Outcome Measures

Phase	Assessment	V1 (Baseline)	V2 (Day 7)	V3 (Day 18)	V4 (Week 4)	V5 (Week 8)	V6 (Week 12)	V7 (Week 24)
Screening	Informed consent & eligibility screening	✓						
	Demographics	✓						
	Dietary and lifestyle information	✓						
Clinical Assessments	Physical examination & vital signs	✓			✓	✓	✓	✓
	12-lead ECG	✓			✓	✓	✓	✓
	Laboratory tests (CBC, liver/renal, lipid, fasting insulin)	✓			✓	✓	✓	✓
	Concomitant medications & adherence	✓	✓	✓	✓	✓	✓	✓

Phase	Assessment	V1 (Baseline)	V2 (Day 7)	V3 (Day 18)	V4 (Week 4)	V5 (Week 8)	V6 (Week 12)	V7 (Week 24)
Primary Outcome	PANSS total score change	✓			✓	✓	✓	✓
Secondary Outcomes	MCCB cognitive function	✓					✓	✓
	CGI	✓	✓		✓	✓	✓	✓
	TESS	✓	✓		✓	✓	✓	✓
	GSRS	✓	✓		✓	✓	✓	✓
	BSFS	✓	✓		✓	✓	✓	✓
Safety Monitoring	AE / SAE reporting	✓	✓		✓	✓	✓	✓
Biospecimen Collection	Blood	✓		✓	✓	✓	✓	✓
	Feces	✓		✓	✓	✓	✓	✓

366 Note: CBC, complete blood count; ECG, electrocardiogram; PANSS, Positive and Negative
367 Syndrome Scale; MCCB, MATRICS Consensus Cognitive Battery; CGI, Clinical Global
368 Impression; TESS, Treatment Emergent Symptom Scale; GSRS, Gastrointestinal Symptom

Rating Scale; BSFS, Bristol Stool Form Scale; AE, adverse event; SAE, serious adverse event.

3.6.6 Outcome Measurement Quality Assurance

To ensure the quality and reliability of outcome measurements, the following procedures will be implemented. All clinical assessors (psychiatrists and psychologists) responsible for administering PANSS, MCCB, CGI, TESS, GSRS, and BSFS will undergo centralized training and certification prior to study initiation. The training program will include didactic instruction on scale administration, video-based case vignettes, and supervised practice with standardized patients. Inter-rater reliability will be established across all participating sites, with an intraclass correlation coefficient (ICC) $\geqslant$ 0.85 required for PANSS total score and MCCB domain scores before study commencement; site investigators failing to meet this threshold will receive additional training until the criterion is achieved. To maintain reliability throughout the trial, joint rating sessions will be conducted quarterly via videoconference, with random double-scoring of assessments (at least 10% of all visits) to monitor drift. For MCCB cognitive assessments, all test administrators will be trained to the same standardized procedures, with strict adherence to the MATRICS standardized administration manual. For adverse event reporting, site investigators will receive training on CTCAE grading criteria and SAE reporting timelines. Assessment windows will be maintained within $\pm$3 days of the scheduled visit dates, with deviations recorded and reported. All outcome data will be entered into the electronic case report form (eCRF) by trained research coordinators, with

source document verification and range checks for data consistency.

3.7 Biospecimen Collection and Laboratory Assays

Fecal and blood samples will be collected at multiple timepoints as specified in **Table 5**. Fecal samples will be aliquoted into three fractions for (1) metagenomics (shotgun sequencing for species/strain profiling and MAG reconstruction), (2) metatranscriptomics (RNA sequencing for active functional expression), and (3) targeted/untargeted metabolomics (UHPLC-HRMS and LC-MS/MS for SCFAs, bile acids, and tryptophan metabolites). Culturomics will be performed from raw fecal aliquots for live strain isolation.

Blood samples will be separated into plasma, serum, and PBMCs. Plasma will be analyzed by Olink/Luminex inflammatory proteomics and metabolomics; serum by clinical biochemistry and immunoglobulin profiling; PBMCs by spectral flow cytometry (≥40 markers), scRNA-seq (10x), bulk RNA-seq, and functional assays (PMA/ionomycin stimulation). A comprehensive summary of biospecimen types, preservation methods, analytical platforms, and targets is provided in **Table 6**. Detailed protocols for each assay are described in the **Supplementary Materials 1**.

Table 6 Biospecimen Types and Analytical Methods

Specimen	Preservation	Assay	Target
Feces (RNA)	RNA stabilization	Metatranscriptomics (Illumina NovaSeq)	Active functional expression (KEGG/MetaCyc/GO)

	buffer		
Feces (DNA)	DNA stabilization buffer	Shotgun metagenomics (Illumina NovaSeq)	Species/strain composition, functional genes, MAG reconstruction, StrainPhlAn4 tracking
Feces (raw)	No additive	Targeted + untargeted metabolomics (UHPLC-HRMS, LC-MS/MS)	SCFAs, bile acids (>20 species), tryptophan metabolites
Feces (raw)	No additive	Culturomics (MALDI-TOF / WGS)	Live bacterial isolation and identification
Plasma	−80°C	Olink/Luminex inflammatory proteomics	Inflammatory cytokines (IL-6, TNF-α, IFN-γ, etc.)
Plasma	−80°C	Metabolomics (LC-MS/MS, UHPLC-HRMS)	SCFAs, bile acids, tryptophan metabolites
Serum	−80°C	ELISA / multiplex assays	IgG/IgA/IgM subclasses, ANA, anti-dsDNA
Serum	−80°C	Automated biochemistry	Liver/renal function, glucose-lipid profile, hs-CRP
PBMC	−80°C	Spectral flow cytometry (≥40 markers)	T/NK/monocyte/B cell subsets, activation markers

PBMC	−80°C	scRNA-seq (10x Genomics)	Single-cell transcriptomics
PBMC	−80°C	Bulk RNA-seq	Global gene expression
PBMC	−80°C	Functional assays (PMA/ionomycin)	Intracellular cytokines (IFN-γ, TNF-α, IL-2, IL-17A)

Note: All biospecimens are stored at −80° C unless otherwise specified. Preservation methods listed refer to the conditions applied immediately after collection prior to storage. SCFAs, short-chain fatty acids; MAG, metagenome-assembled genome; WGS, whole-genome sequencing; PBMC, peripheral blood mononuclear cells; ANA, antinuclear antibodies; hs-CRP, high-sensitivity C-reactive protein.

4. Data Analysis Plan

4.1 Statistical analysis of clinical outcomes

Primary analysis: The primary endpoint is the change in PANSS total score during follow-up. Intention-to-treat (ITT) analysis with the last observation carried forward (LOCF) method will handle missing data. Analysis of covariance (ANCOVA) will compare group differences at each time point, adjusting for baseline values, BMI, age, sex, disease duration, and other covariates. The primary contrast will be the between-arm difference in PANSS total score change from baseline to week 24, reported with 95% confidence interval and Cohen's d. The intention-to-treat (ITT) set will serve as the primary analysis population, with per-protocol (PP) analysis as

sensitivity. And then the clinical outcomes will be analyzed using the same linear mixed model framework: PANSS subscales (positive, negative, general), response rate (≥30% PANSS reduction, modified Poisson regression), and remission rate (Andreasen criteria, Fisher's exact test). Time course of PANSS will be visualized as arm × visit interaction plots with 95% CIs.

Secondary analyses: (1) Mixed-effects models for repeated measures (MMRM) will assess changes in MCCB scores and microbiota diversity over time; (2) Pearson or Spearman correlation analyses will examine associations between microbiota diversity and PANSS/MCCB scores; (3) Chi-square or Fisher's exact tests will be used for safety outcomes(17). Safety outcomes (adverse events by CTCAE grade and system organ class) will be compared between arms using Fisher's exact test. Laboratory parameters will be analyzed using the same LMM as the primary analysis. All statistical analyses will be performed using R 4.3 (lme4 package) or SPSS 26.0. No interim analyses are planned unless required by the Data Monitoring Committee for safety review.

To characterize FMT-induced alterations in gut microbial ecology, shotgun metagenomic sequencing data will be processed using MetaPhlAn 4 for taxonomic profiling. Alpha diversity will be assessed using the Shannon diversity index, observed species richness, and Pielou's evenness, with between-group comparisons performed using Wilcoxon rank-sum tests and longitudinal changes evaluated by linear mixed-effects models. Beta diversity will be calculated using Bray-Curtis dissimilarity and Aitchison distance, with principal coordinate analysis (PCoA) for

visualization and PERMANOVA (adonis2) for statistical testing of community structure differences between groups. Differential abundance analysis of species, gene families, and metabolic pathways will be performed using DESeq2 or MaAsLin2, adjusting for baseline covariates and multiple testing (false discovery rate < 0.2). Temporal dynamics of specific taxa of interest (e.g., short-chain fatty acid producers, Bifidobacterium species) will be examined using generalized estimating equations to account for within-subject correlations across repeated measures.

4.2 Capsule Fidelity

To evaluate, at the manufacturing-batch level, the degree to which the FMT capsule retains the original donor stool microbiota, capsule–stool Aitchison distance will be calculated. This is a quality-control step, not an engraftment analysis. Fidelity assessment provides context for interpreting apparent engraftment failures, taxa absent post-FMT may have been lost during capsule preparation rather than failing to colonize. Capsule–stool Aitchison distance is included as a candidate predictor in the engraftment determinants model, under the hypothesis that lower manufacturing fidelity predicts lower donor strain engraftment.

4.3 Engraftment and Statistical Analysis Plan

4.3.1 Microbiome Engraftment and Strain-Level Dynamics

To evaluate donor microbiota colonization efficiency post-FMT, strain-level profiling will be performed using StrainPhlAn 4, which infers strain identity through phylogenetic analysis of species-specific marker genes. Species-level metagenomic profiling will be conducted using MetaPhlAn 4(18). The definitions, formulas, and

applications of all engraftment metrics are summarized in **Table 7**. Detailed contents for each assay are described in the **Supplementary Materials 2**. The phylogenetic distance threshold for strain assignment will be determined using Youden's index(19, 20).

Table 7 Engraftment Metrics Summary

Metric	Definition	Formula	Notes	Application
Bray-Curtis (BC) Similarity	Community similarity based on relative abundances	$BC_{IJ} = \frac{\sum_{k=1}^{p} \lvert x_{ik} - x_{jk} \rvert}{\sum_{k=1}^{p} (x_{ik} + x_{jk})}$ $Bray - Curtis\ Similarity = 1 - BC_{ij}$	x_{ik} and x_{jk} are the relative abundances of species k in samples i and j, and p is the total number of species	Assess compositional convergence between donor and recipient
Donor Similarity Index (DSI)	Normalized similarity to donor relative to baseline	$DSI_t = \frac{Sim(R_t, D) - Sim(R_0, D)}{1 - Sim(R_0, D)}$	R_0 is the recipient's baseline microbiota, R_t is the recipient's microbiota at post-FMT timepoint t, D is the donor's microbiota, and $Sim(.)$ is any chosen similarity metric (e.g., Bray-Curtis Similarity)	Track longitudinal shift toward donor profile

Jaccard Index (J)	Presence/absence-based species overlap	$J(A,B)=\frac{\lvert A\cap B\rvert}{\lvert A\cup B\rvert}$	A and B are the sets of species (or strains) detected in two samples (e.g., donor and post-FMT recipient)	Assess species-level donor strain engraftment
Aitchison Distance (AD)	CLR-transformed functional distance	$AD(x,y)=\sqrt{\sum_{k=1}^{p}\left[\ln(\frac{x_k}{g(x)})-\ln(\frac{y_k}{g(y)})\right]^2}$	x and y are composition vectors (e.g., functional profiles), and $g(x)=(\prod_{k=1}^{p}x_k)^{1/p}$ is the geometric mean of the composition	Evaluate functional vs taxonomic engraftment
Microbiota Compatibility Index (MCI)	Donor–recipient compatibility score (0–100)	$MCI=\alpha\cdot P+\beta\cdot F+\gamma\cdot M$	P represents phylogenetic overlap between donor and recipient, F represents functional gene repertoire similarity, and M represents metabolic pathway complementarity.	Integrate phylogenetic, functional, and metabolic alignment

Strain Engraftment Rate (SER)	Proportion of donor strains detected post-FMT	$SER = \frac{N_{donor\ strains\ detected\ post-FMT}}{N_{donor\ strains\ profiled}}$	The weights α, β, γ are empirically determined, with $\alpha + \beta + \gamma = 1$ $N_{donor\ strains\ profiled}$ is the total number of donor strains for which strain-level profiling is feasible, and $N_{donor\ strains\ detected\ post-FMT}$ is the number of these donor strains successfully identified in the recipient after transplantation	Primary measure of donor colonization efficiency
Strain-Sharing Rate (SSR)	Shared strains between two samples	$SSR(A,B) = \frac{N_{shared\ strains\ between\ A\ and\ B}}{N_{species\ profiled\ in\ common\ between\ A\ and\ B}}$	$N_{shared\ strains}$ is the number of strains detected in both samples A and B, and $N_{species\ profiled\ in\ common}$ is the number of species for which	Compare donor–post, pre–post, and donor–pre pairs

			StrainPhlAn provided strain-level information in both samples	
Fraction of Donor Strains (FDS)	Donor-derived proportion of post-FMT strain pool	$FDS = \frac{N_{shared\ strains\ (post-FMT\ vs.\ donor)}}{N_{strains\ profiled\ in\ post-FMT}}$	FDS represents the proportion of the post-FMT recipient's strain pool that originated from the donor	Quantify donor contribution to recipient microbiome
Fraction of Retained Strains (FRS)	Baseline-derived proportion of post-FMT strain pool	$FRS = \frac{N_{shared\ strains\ (post-FMT\ vs.\ pre-FMT)}}{N_{strains\ profiled\ in\ post-FMT}}$	FRS represents the proportion of the post-FMT strain pool that persisted from the recipient's baseline	Quantify persistence of recipient baseline strains

Bray-Curtis dissimilarity quantifies compositional differences based on relative abundances and will be applied to donor-post-FMT, pre-FMT-post-FMT, and donor-pre-FMT pairs. Donor Similarity Index normalizes post-transplant similarity to the donor relative to baseline, enabling longitudinal tracking independent of inter-individual variation. Jaccard index measures presence/absence overlap, Aitchison distance assesses functional convergence via centered log-ratio (CLR) transformation, and Microbiota Compatibility Index (scaled 0–100) integrates phylogenetic, functional, and metabolic compatibility between donor and recipient, with scores $\geqslant$ 80 indicating high compatibility, 60–79 moderate compatibility, and <60 low compatibility(19, 21-24). SER is defined as the number of donor-derived strains detected post-FMT divided by the number of donor strains profiled(19). SSR quantifies shared strains between sample pairs, and FDS/FRS represent the donor-derived and baseline-retained proportions of the post-FMT strain pool. For comparison with published literature, we will additionally calculate the species-level engraftment rate without subtracting recipient-baseline strains.

We will explore donor-related (e.g., α-diversity, specific taxa abundance), recipient-related (e.g., baseline diversity, immune status), and procedural (e.g., bowel preparation) factors influencing engraftment using generalized linear models, adjusted for potential confounders. Candidate predictors for the engraftment determinants model will include: (1) Donor-related: donor alpha diversity, Bifidobacterium abundance, proportion of high-engraftment phyla (Bacteroidetes, Actinobacteria); (2) Recipient-related: pre-FMT alpha diversity (Shannon), pre-FMT beta distance to

497 donor, antibiotic-free interval, antipsychotic duration; (3) Capsule fidelity: capsule –
498 stool Aitchison distance; (4) Procedural: cumulative FMT dose, administration
499 frequency. The modeling strategy will proceed in three stages: (1) linear regression
500 with all candidate predictors; (2) Random Forest for feature importance ranking; (3)
501 LASSO regression for a parsimonious final model.

502 4.3.2 Engraftment–clinical association

503 We will examine the relationship between engraftment metrics and clinical
504 outcomes using multiple approaches: (1) Spearman correlation between SER and
505 ΔPANSS, adjusted for baseline PANSS and antipsychotic class via linear regression;
506 (2) comparison of ΔPANSS between high-SER and low-SER groups (upper vs lower
507 tertile) using Welch's t-test or Mann–Whitney test, and response rate via χ^2 test; (3)
508 Jonckheere–Terpstra test for trend across SER tertiles; and (4) LASSO regression
509 selecting engrafted species as predictors of ΔPANSS to identify species-level
510 contributions to clinical improvement.

511 4.3.3 Predictive Modeling of Post-FMT Microbiome Composition

512 Input features for species presence/absence prediction will include: donor species
513 abundance, recipient baseline species abundance, species prevalence in healthy
514 reference populations, and taxonomic affiliation. Model performance will be
515 evaluated by 5-fold cross-validation and leave-one-donor-out validation, with
516 AUROC as the primary metric(19, 20). For strain-level colonization prediction,
517 LASSO regression will incorporate: recipient baseline species richness,
518 donor-to-recipient species abundance ratio (propagule pressure), donor–recipient

strain population diversity, and phylogenetic distance(20).

Magnitude of Donor Colonization (MDC) and Loss of Baseline SGBs (Species-Level Genome Bins): The magnitude of donor colonization will be calculated as the sum of relative abundances of all donor-derived strains in the post-FMT recipient. SGBs detected in the recipient at baseline but no longer detectable post-FMT will be classified as "lost"(25). The proportion of lost SGBs will be calculated as the number of lost SGBs divided by the total number of SGBs present at baseline.

$$MDC = \sum_{i \in S_{shared}} RA_{i,\ post-FMT}$$

S_{shared} is the set of donor-derived strains detected in the post-FMT recipient, and $RA_{i,\ post-FMT}$ is the relative abundance of strain i in the post-FMT recipient sample.

$$\Pr oportion of\ lost\ SGBs = \frac{N_{SGBs\ lost}}{N_{SGBs\ present\ at\ baseline}}$$

$$N_{SGBs\ lost} = \left| SGB_{baseline} \setminus SGB_{post-FMT} \right|$$

$SGB_{baseline}$ is the set of SGBs detected in the recipient at baseline, and $SGB_{post-FMT}$ is the set of SGBs detected post-FMT. The operator \ denotes set difference (elements present in the first set but not in the second).

4.4 Multi-Omics Integrative Analysis

To systematically elucidate the mechanisms linking FMT-induced microbial changes to clinical outcomes, an integrated multi-omics analysis framework will be implemented across five complementary layers: (1) microbiome layer (fecal DNA): metagenomic species/strain composition and functional gene repertoire; (2) functional activity layer (fecal RNA): metatranscriptomic active pathway expression; (3)

metabolite layer (fecal raw and plasma): targeted and untargeted metabolomics of SCFAs, bile acids, and tryptophan metabolites; (4) immune response layer (plasma + PBMC): inflammatory proteomics, high-dimensional immunophenotyping, and PBMC functional assays; and (5) host transcriptome layer (PBMC bulk RNA): whole-blood gene expression profiling.

Integration across these layers will be performed using: (i) Spearman correlation to assess pairwise associations among microbiota, metabolites, immune factors, and clinical parameters; (ii) mediation analysis (mediation R package, 1,000 bootstrap iterations) to construct "microbiota-metabolite-immune-clinical" mediation models, reporting the average causal mediation effect (ACME); (iii) DIABLO (Data Integration Analysis for Biomarker discovery using Latent components) from the mixOmics package for multi-block integration; and (iv) canonical correspondence analysis (CCA) to assess associations between microbial communities and metabolite profiles. This five-layer integrative strategy enables systematic mapping of the "microbiota-metabolite-immune-clinical" landscape, consistent with the multi-omics methodological frameworks established in recent FMT trials(19, 20, 26, 27). The five-layer integrative framework, including data sources, analytical platforms, and targets for each layer, is summarized in **Table 8**.

Table 8 Multi-Omics Integrative Analysis Framework

Layer	Data Source	Technology	Analytical Target

Layer 1: Microbiome	Fecal DNA	Shotgun metagenomics (Illumina NovaSeq)	Species/strain composition, functional gene repertoire
Layer 2: Functional Activity	Fecal RNA	Metatranscriptomics (Illumina NovaSeq)	Active pathway expression
Layer 3: Metabolome	Fecal raw / plasma	Targeted / untargeted metabolomics (UHPLC-HRMS, LC-MS/MS)	SCFAs, bile acids (>20 species), tryptophan metabolites
Layer 4: Immune Response	Plasma / PBMC	Inflammatory proteomics (Olink/Luminex), spectral flow cytometry (≥40 markers), functional assays	Cytokine profiles, immune cell subsets, T-cell function
Layer 5: Host Transcriptome	PBMC	Bulk RNA-seq	Global gene expression (immune/mitochondrial pathways)
Integration Methods		Spearman correlation, mediation analysis, DIABLO, CCA	Map "microbiota–metabolite–immune–clinical" landscape

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4.5 Sensitivity & Supplementary Analyses

To assess the robustness of the primary and secondary findings, a series of sensitivity and supplementary analyses will be performed as summarized in **Table 9**.

Table 9 Sensitivity and Supplementary Analyses

Analysis	Method	Purpose
Per-protocol analysis	Exclude major protocol violators (e.g., <80 capsules consumed, prohibited medications)	Assess robustness of ITT findings
Subgroup analyses	Stratify by sex, age (<40 vs ≥40 years), disease duration (<5 vs 5–10 years), antipsychotic class (olanzapine-based vs others), and baseline PANSS severity (median split); test interaction terms in LMM	Identify potential effect modifiers
Dietary adjustment	Include FFQ data as covariate in LMM (if collected)	Control for dietary confounding
Engraftment threshold sensitivity	Test alternative SER cut-offs (median, quartiles)	Validate robustness of high-vs-low engraftment comparisons
Missing data	MICE vs completers-only analysis	Evaluate impact of missing data on outcomes

Notes: ITT, intention-to-treat; LMM, linear mixed model; FFQ, food frequency questionnaire;

SER, strain engraftment rate; MICE, multiple imputation by chained equations.

5. Ethics and Compliance

5.1 Ethical Approval

This study protocol has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (No: XJTU1AF2022LSK-435-01) and the ethics committees of all participating centers. The trial will be conducted in accordance with the principles of the Declaration of Helsinki (Fortaleza 2013 revision) and the International Conference on Harmonisation Good Clinical Practice (ICH-GCP E6 R2) guidelines.

5.2 Protocol Amendments

No changes have been made to the trial protocol since its commencement. The protocol described herein represents the final approved version. Any modifications to the study protocol that may affect the scientific integrity, participant safety, or conduct of the trial will be formally documented and submitted for review and approval by the lead Ethics Committee and all participating site ethics committees prior to implementation. Substantial amendments will also be updated in the *ClinicalTrials.gov* registry (NCT05850585) in accordance with regulatory requirements, and all amendments will be reported in the final trial publication, including the nature of the change, the date of amendment, and the rationale.

5.3 Informed Consent

Written informed consent will be obtained from all participants or their legally

authorized representatives (LARs) prior to enrollment. For participants with impaired decision-making capacity at screening, consent will be obtained from their LARs, and re-consent will be sought from the participant personally as soon as their decisional capacity is restored to the extent permitted by clinical evaluation. All participants (or their LARs) will be informed of the study's purpose, procedures, potential risks, benefits, and their right to withdraw from the study at any time without penalty or loss of standard medical care.

5.4 Data Protection and Confidentiality

All participant-identifiable information will be anonymized and replaced with unique coded identifiers. The electronic database will be password-protected, encrypted, and accessible only to authorized research personnel directly involved in the trial. Data will be retained for a period of 10 years after trial completion at the lead center's secure server, in compliance with national data protection regulations, and will be destroyed thereafter in accordance with the approved data management plan. Secondary use of anonymized data for future research purposes will require separate ethics approval and, where applicable, re-consent from participants.

5.5 Data Monitoring

An independent Data Monitoring Committee (DMC), comprising clinical experts, biostatisticians, and an ethicist, will review cumulative safety data at predefined intervals (every 3 months) throughout the trial. The DMC may recommend early termination or protocol modification if unacceptable safety signals emerge.

5.6 Trial Registration

This trial has been prospectively registered with the U.S. National Institutes of Health *ClinicalTrials.gov* database (registration number: NCT05850585). The results will be disseminated through peer-reviewed publications regardless of the outcome direction, and will be made publicly available in accordance with the International Committee of Medical Journal Editors (ICMJE) data-sharing requirements.

5.7 Patient and Public Involvement

Patients and the public were not involved in the design, conduct, or reporting of this trial protocol. The trial was designed by academic investigators based on preclinical evidence and prior clinical observations. However, the findings of this trial will be disseminated through peer-reviewed publications and presented at patient advocacy group meetings to ensure that results are accessible to the schizophrenia community. Patient representatives will be consulted for the development of patient-friendly summaries of the trial results upon study completion.

6. Discussion

Schizophrenia treatment faces the dual challenges of limited efficacy and significant adverse effects. The gut microbiota, through the microbiota-gut-brain axis, influences brain function and behavior, providing a novel therapeutic target for schizophrenia(28, 29). FMT has demonstrated efficacy in recurrent *Clostridium difficile* infection and certain gastrointestinal disorders, and has shown preliminary positive effects in autism spectrum disorder, yet its role in schizophrenia remains unclear(14). This trial addresses the gap by providing a rigorous evaluation of FMT as

an adjunctive therapy in non-treatment-resistant schizophrenia.

A critical determinant of FMT efficacy that warrants prospective evaluation is the degree of donor microbiota engraftment, the extent to which transplanted strains establish and persist in the recipient. Engraftment is shaped by donor-related factors (microbiota diversity, diet, and phylogenetic compatibility)(19, 24), recipient-related factors (baseline microbial diversity, immune status, and "gatekeeper" species such as *Bacteroides uniformis* and *Alistipes shahii*)(30), and procedural variables (bowel preparation, route, and dosing frequency)(19). Recipient factors, particularly pre-treatment dysbiosis characterized by reduced species richness, consistently outweigh donor factors in driving strain-level outcomes, suggesting that patients with more disrupted microbiomes may derive greater benefit(19, 24). Meta-analytic evidence supports a positive association between higher donor strain engraftment and clinical success ($P = 0.017$ across 24 studies)(19), although the heterogeneity of FMT responses across diseases underscores the need for disease-specific evaluation(23, 30). Strain-level engraftment patterns, including donor colonization, recipient persistence, and coexistence, may further refine outcome prediction(20), and machine learning classifiers using baseline donor-recipient features have achieved an average AUROC of 0.77 in predicting post-FMT species presence(19).

Conducting this trial across 11 centers presents several practical challenges. First, recruitment of non-treatment-resistant schizophrenia patients requires coordinated efforts across sites to ensure steady enrollment within the 18-month window. Early engagement with community mental health services and clear communication of the

trial’s adjunctive nature (standard antipsychotic treatment continues unchanged) will be essential to address potential participant concerns. Second, maintaining adherence to the 120-capsule regimen (10 capsules/day for 6 days, then 5 capsules/day for 12 days) requires robust monitoring. Third, the integrity of blinding depends on the visual and olfactory indistinguishability of FMT and placebo capsules, which is ensured by a single third-party manufacturer. Fourth, biospecimen handling, particularly fecal sample collection and storage at −80° C across multiple sites, requires standardized protocols for collection, aliquoting, and transport to maintain sample integrity for metagenomic, metatranscriptomic, and metabolomic analyses. Site staff will receive centralized training on these procedures.

The present trial is uniquely positioned to address these engraftment-related questions through its integrated multi-omics framework and strain-level tracking using StrainPhlAn 4. By systematically linking baseline microbial features, engraftment metrics (Bray-Curtis, DSI, Jaccard, Aitchison, MCI, SER, SSR, FDS/FRS), and clinical outcomes, this study will: (i) identify predictive biomarkers of FMT response in schizophrenia; (ii) elucidate whether engraftment magnitude correlates with PANSS improvement, MCCB cognitive gains, or metabolic changes; and (iii) generate hypotheses for personalized donor-recipient matching strategies. This mechanistic depth distinguishes the present trial from existing FMT studies in psychiatry, which have largely employed descriptive microbiome profiling without strain-level resolution.

The novelty of this trial lies in three aspects. First, it is the first multicenter RCT to

evaluate FMT specifically in non-treatment-resistant schizophrenia, complementing the ongoing TRS-focused trial (ChiCTR2400092799) and addressing a substantially larger patient population. Second, the integration of multidimensional data, clinical symptoms, cognitive function (MCCB), gut metagenomics/metabolomics, inflammatory proteomics, and high-dimensional immunophenotyping, enables systematic exploration of the microbiota-immune-cognition pathway. Third, the use of uniformly prepared FMT capsules by a third-party manufacturer ensures intervention standardization and reproducibility(31).

Compared with the published TRS-FMT protocol (ChiCTR2400092799), the present trial offers complementary advantages: a two-arm design providing greater statistical power for the primary PANSS endpoint, exclusive use of oral capsules for superior scalability and patient acceptability, systematic cognitive assessment via MCCB, comprehensive metabolic monitoring, and a 24-week follow-up to assess durability. While the TRS protocol addresses a high-unmet-need population, the present study targets a broader group where earlier intervention may prevent treatment resistance from consolidating.

Several limitations should be acknowledged. First, patients with schizophrenia often present with multiple comorbidities and complex medication regimens, which may confound the efficacy evaluation of FMT. Second, long-term efficacy and safety beyond 24 weeks require further validation through extended follow-up studies(17). Third, donor-recipient matching strategies have not been systematically implemented in this trial, and the multi-omics analyses are inherently exploratory. Findings will

require independent replication before clinical translation(13).

Looking forward, the results of this trial will inform several key areas for future investigation. If engraftment metrics correlate with clinical response, subsequent studies could prospectively test donor selection algorithms to maximize colonization efficiency. The multi-omics data may reveal specific microbial taxa, metabolites, or immune signatures that mediate therapeutic effects, potentially guiding the development of next-generation microbiome therapeutics (e.g., defined bacterial consortia or postbiotics). Finally, the durability of FMT effects will determine whether maintenance dosing schedules are needed, an question that the 24-week follow-up is designed to preliminarily address.

In conclusion, this trial will provide high-level evidence on the efficacy and safety of FMT as an adjunctive therapy for non-treatment-resistant schizophrenia, contributing to the evidence base for microbiome-based interventions in psychiatric disorders and informing the design of future implementation studies.

Abbreviations

ACME	Average Causal Mediation Effect
ANCOVA	Analysis of Covariance
BSFS	Bristol Stool Form Scale
CCA	Canonical Correspondence Analysis
CFU	Colony-Forming Units

CGI	Clinical Global Impression
CTCAE	Common Terminology Criteria for Adverse Events
DIABLO	Data Integration Analysis for Biomarker discovery using Latent components
FDS	Fraction of Donor Strains
FMT	Fecal Microbiota Transplantation
FRS	Fraction of Retained Strains
GSRS	Gastrointestinal Symptom Rating Scale
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
ITT	Intention-to-Treat
LASSO	Least Absolute Shrinkage and Selection Operator
LMM	Linear Mixed Model
MCCB	MATRICS Consensus Cognitive Battery
MICE	Multiple Imputation by Chained Equations
PANSS	Positive and Negative Syndrome Scale
PP	Per-Protocol
RF	Random Forest
SER	Strain Engraftment Rate

SGBs	Species-Level Genome Bins
SSR	Strain-Sharing Rate
TESS	Treatment Emergent Symptom Scale

Declarations

Ethics approval and consent to participate

This study protocol has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Approval No. XJTU1AF2022LSK-435-01) and the ethics committees of all participating centers. The additional movie files show this in more detail (see **Additional file 1 and 2**). The trial will be conducted in accordance with the principles of the Declaration of Helsinki (Fortaleza 2013 revision) and the International Conference on Harmonisation Good Clinical Practice (ICH-GCP E6 R2) guidelines. Written informed consent will be obtained from all participants or their legally authorized representatives prior to enrollment. For participants with impaired decision-making capacity at screening, consent will be obtained from their legally authorized representatives, and re-consent will be sought from the participant personally as soon as their decisional capacity is restored. All participants (or their legally authorized representatives) will be informed of the study's purpose, procedures, potential risks, benefits, and their right to withdraw from the study at any time without penalty or loss of standard medical care.

Consent for publication

Written informed consent will be obtained from all participants or their legally authorized representatives (LARs) prior to enrollment (see **Additional file 3**).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

CW is the principal investigator of the grant; **CW, YL, MJ and JL** conceptualized the study. **CW, XM, BH, MJ, YJ, and YZ** adapted the intervention and developed the implementation strategies. **RG, XS, XM, and FZ** contributed to the refinement of the implementation strategies. **CW** drafted the manuscript, **YJ, MJ, XM, BH, MJ, YJ, YZ, RG, XS, XM, and FZ** revised it. All authors (**CW, YJ, MJ, XM, BH, MJ, YJ, YZ, RG, XS, XM, and FZ**) critically reviewed the manuscript for intellectual content and approved the final version.

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CONSORT Checklist

This protocol is reported in accordance with the CONSORT 2025 Statement for randomized controlled trials. A completed CONSORT 2025 checklist is provided in **Additional file 6** (items pertaining to results will be completed at trial conclusion). The trial was prospectively registered at *ClinicalTrials.gov* (NCT05850585) prior to participant enrollment.

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Figure Legend

Figure 1. CONSORT flow diagram of the trial design.

Eligible patients with non-treatment-resistant schizophrenia (PANSS total score ≥ 60, disease duration ≤ 10 years) will be randomized 1:1 to receive either FMT capsules or placebo

capsules (identical in appearance, weight, and odor, containing lyophilized protectant only) adjunctive to standard antipsychotic treatment, with both groups following the same administration schedule. Follow-up assessments are scheduled at baseline (V1), day 7 (V2), day 18 (V3, end of intervention), week 4 (V4), week 8 (V5), week 12 (V6), and week 24 (V7, endpoint). Assessments include clinical scales (PANSS, MCCB, TESS, CGI, GSRS, BSFS), laboratory examinations (blood counts, liver/renal function, lipid profile, fasting insulin), and biospecimen collection (blood and feces) for multi-omics profiling. The analytical framework comprises four layers: Layer 1 evaluates the clinical efficacy of FMT versus placebo (primary and secondary outcomes). Layer 2 assesses capsule fidelity by comparing the finished capsule microbiota to the original donor stool. Layer 3 quantifies donor strain engraftment, identifies its determinants, and examines its association with clinical outcomes. Layer 4 integrates multi-omics data (microbiome, metabolome, immune, and host transcriptome) to explore mechanistic pathways. Clinical scale abbreviations: FMT, fecal microbiota transplantation; PANSS, Positive and Negative Syndrome Scale; MCCB, MATRICS Consensus Cognitive Battery; TESS, Treatment Emergent Symptom Scale; CGI, Clinical Global Impression; GSRS, Gastrointestinal Symptom Rating Scale; BSFS, Bristol Stool Form Scale. Statistical and analytical abbreviations are defined in the main text abbreviations table.

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