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Donor Selection for Fecal Microbiota Transplantation: Current Criteria, Safety and Future Perspectives - A Narrative Review

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1. Introduction

Fecal microbiota transplantation (FMT) is a therapeutic method that involves modifying the composition of the gut microbiome by transferring fecal material from a healthy donor [5]. The best-documented indication for its use is recurrent Clostridioides difficile infection (rCDI) in children and adults, in which the efficacy of FMT exceeds 90% [2,5]. In this patient group, the therapy is a primary treatment option for both subsequent recurrences and cases resistant to standard antibiotic therapy. A clinical response is defined as an improvement in the frequency and consistency of bowel movements [10].

According to the current guidelines of the American Gastroenterological Association (AGA), FMT is most often recommended after at least a second recurrence of infection, while in some European countries, including Poland, it is recommended after the second episode of the disease [13]. In addition to the primary indications, this method is used experimentally in inflammatory bowel disease, irritable bowel syndrome, and other gastrointestinal disorders [11,13], and potentially also in metabolic and neuropsychiatric disorders [13].

The increasing popularity of FMT comes from the expansion of its clinical applications; however, donor selection remains a key factor determining the therapy's effectiveness. At the same time, the literature highlights the lack of global standardization of procedures and significant differences in donor eligibility criteria, which makes it difficult to compare study results and apply consistent clinical standards. Therefore, this study aims to review current donor selection criteria and analyze the factors influencing the efficacy and safety of FMT.

2. The Importance of the Donor in the Mechanism of Action of FMT

Fecal microbiota transplantation (FMT) is a procedure whose effectiveness depends on the interaction between the donor material and the recipient's system, and the microbiota can be regarded as a "living drug" capable of temporarily colonizing the gut. Immediately after the procedure, a temporary change in the recipient's microbiota toward the donor's profile is observed; however, over time, there is a partial return to the baseline state [2]. The taxonomic composition of the donor microbiota is identified as one of the key factors determining the success of the therapy [3], which underscores the importance of microbial diversity and the interaction between the donor and the recipient.

An important factor influencing the effectiveness of FMT is the donor's clinical characteristics. Antibiotic use within 12 months prior to donation is associated with reduced treatment efficacy, which results from long-lasting antibiotic-induced disturbances in the gut microbiota; these disturbances may last up to a year [1]. Furthermore, the consistency of the donor's stool

correlates with the clinical outcome; looser stools have been associated with better treatment results [1]. This correlation may reflect differences in the structure of the microbiota and dominant enterotypes, including a higher prevalence of the Prevotella enterotype in samples with a softer consistency [1].

The mechanism of action of FMT in inflammatory bowel diseases is not fully understood, but it is believed that this therapy may correct dysbiosis, leading to a decrease or reduction in inflammation [5]. FMT affects not only bacteria but also viruses, fungi, protists and archaea, as well as their metabolites, which highlights its complex effects [5].

The heterogeneity of donors remains a problem, as it may affect treatment outcomes. It has been suggested that FMT failures may result from the selection of a less effective donor, rather than from the ineffectiveness of the method itself [9]. A multicenter study demonstrated that excluding low-efficacy donors and using a more intensive FMT regimen increased the cure rate for Clostridioides difficile [14]. Additionally, physical characteristics of the donor material, such as stool moisture content, may promote more effective colonization of the recipient's intestine [15]. In summary, the data indicate that the donor profile can significantly influence the efficacy of FMT, which explains its key role in the mechanism of the therapy.

3. Current guidelines for donor selection

The current approach to fecal microbiota transplantation (FMT) is regulated by recommendations from scientific societies, such as the AGA and ESGE, and by the Food and Drug Administration (FDA)'s regulatory framework, which classifies FMT as a biological product and an investigational drug, except for its use in recurrent Clostridioides difficile infections, where a discretionary approach is applied [5].

Donor eligibility criteria include a range of demographic and clinical characteristics based on guidelines from various centers. Younger individuals, typically under 50 years of age, are preferred, as aging is associated with changes in the composition of the microbiota [2,4]. Some protocols specify an age range of 18–65 years and a Body Mass Index (BMI) of 18–28 kg/m², while a BMI above 30 is an exclusion criterion due to potential microbiota disturbances associated with obesity and metabolic disorders [1,2]. A minimum of three months without antibiotic therapy prior to donation is also required and donors should be able to provide multiple samples over an extended period [1,4].

Previously, related donors, such as family members or spouses, were preferred, based on the assumption of greater microbiota similarity and potential immunological tolerance [11]. Currently, though, clinical data indicate that the efficacy of FMT does not depend significantly

on the relationship between the donor and the recipient, and meta-analyses show no advantage of related donors over unrelated ones [8,11]. In practice, models based on anonymous, repeatedly screened donors are increasingly used, driven by the desire to enhance safety, standardization, and the availability of biological material.

It is also worth mentioning the lack of a standardised donor qualification tool, despite the use of strategies similar to those for human tissue donors. Contemporary selection systems are therefore based on a multi-stage, rigorous screening of healthy volunteers, aimed at minimizing the risk of transmitting pathogens and increasing the predictability of the clinical outcome of FMT [4,5].

4. Medical history and clinical evaluation of the donor

The FMT donor selection process is based on a detailed clinical history and an assessment of risk factors; data from large stool banks indicate a high rejection rate among candidates - approximately 90% in the United States and 50% in Australia - as early as the initial screening stage [16]. This underscores the crucial role of clinical screening even before laboratory tests are performed.

4.1 Exclusionary conditions

The most common reasons for exclusion include chronic diseases and conditions associated with potential gut dysbiosis, including allergies and gastrointestinal disorders [1]. Individuals with gastrointestinal diseases (inflammatory bowel disease, irritable bowel syndrome, celiac disease, and other chronic gastroenterological conditions), current intestinal symptoms (e.g., diarrhea, blood in the stool), and a significant family history of colorectal cancer and inflammatory bowel disease are also excluded [4,16].

A significant group consists of system diseases, including autoimmune, metabolic (such as obesity, metabolic syndrome, diabetes), neurological and psychiatric conditions [4,16]. In addition, individuals with a history of cancer, including gastrointestinal cancers and polyposis syndromes, are excluded [20].

Key exclusion criteria also include infectious and epidemiological factors: infections with HBV, HCV, HIV, and HTLV; syphilis; active infections; risky behaviors; drug use; recent hospitalizations; high-risk travel to exotic destinations; piercings; tattoos; and recent intestinal infections [16]. Temporary periods of disqualification related to vaccinations and invasive procedures are also taken into account [4].

4.2 Lifestyle

The lifestyle assessment includes a medical history regarding health, habits, and environmental exposures [2]. It is crucial to eliminate factors that affect the microbiota, such as chronic use of antibiotics, immunosuppression, chemotherapy, and proton pump inhibitors [4]. The donor's overall health and lifestyle are also important.

4.3 Risk factors

One of the most important risk factors is exposure to antibiotics, which can reduce the effectiveness of FMT by causing long-term disruptions to the microbiota. It is recommended to avoid antibiotic therapy for at least 3 months prior to donation [1].

Temporal factors are also important, such as recent infections, hospitalizations, travel, invasive procedures, or exposures that increase the risk of pathogen transmission [4]. It is recommended to repeat screening tests regularly every 8-12 weeks and to complete a health questionnaire before each donation to assess safety on an ongoing basis [4].

5. Laboratory tests for the donor

The qualification of an FMT donor requires a comprehensive panel of laboratory tests on blood and stool samples, aimed at ruling out infectious diseases and conditions that could affect the safety and efficacy of the procedure. Donors undergo screening that includes both a clinical history and detailed laboratory tests, including a comprehensive evaluation of blood and stool samples for infectious diseases [2,5]. As standard practice, morphological, biochemical, and serological parameters are analyzed, including markers of viral infections (HAV, HBV, HCV, HEV, HIV), as well as indicators of inflammation and organ function [4,10,13].

5.1 Blood tests

The basic panel includes a complete blood count, liver enzymes (ALT, AST), bilirubin, creatinine and CRP, as well as comprehensive serological testing for hepatotropic viruses and HIV [4,13]. Many protocols also include testing for syphilis, CMV, and EBV infections, as well as HTLV [13]. Depending on the geographic region, the panel may be expanded to include opportunistic pathogens such as Strongyloides stercoralis or Listeria [10].

Another important aspect of safety is monitoring for multidrug-resistant organisms (MDRO), including ESBL, VRE, CRE, and MRSA [12]. The importance of limiting the time between testing and donation - which should not exceed a few weeks (e.g., 21 days) - is also emphasized to reduce the risk of infections during the so-called serological window [11]. More advanced

safety protocols additionally include material quarantine, such as freezing samples and retesting the donor after approximately 90 days [13].

5.2 Stool tests

Stool analysis includes a broad spectrum of pathogens, including antibiotic-resistant bacteria (MRSA, VRE, ESBL, CRE), as well as classic intestinal pathogens such as Salmonella, Shigella, Campylobacter, STEC, and Yersinia [2,4]. Clostridioides difficile, parasites (Giardia, Cryptosporidium, Blastocystis, Dientamoeba), enteric viruses (norovirus, rotavirus, adenovirus), and Helicobacter pylori are also routinely detected [4,13]. The presence of any of these agents constitutes a significant reason for donor exclusion.

5.3 New developments

Molecular and metagenomic methods, which allow for a more precise analysis of the microbiome, are becoming increasingly important in donor quality assessment. An example is the DoMEI (Donor Microbial Evaluation Index), first introduced and described by Jianquan He and co-authors [20]. This index combines data on beneficial and potentially harmful taxons and the overall microbial richness of the sample. This makes it possible to quantitatively assess donor quality based on discrepancies from the reference population.

6. The concept of the “super-donor”

The phenomenon known as the “super-donor” refers to the observation that the effectiveness of FMT may depend significantly on the specific individual donating the material, particularly in inflammatory bowel diseases [21]. In one of the first randomized controlled trials, 39% of patients achieved remission after a transplant from a single donor, while only 10% did so in groups receiving material from other donors, which led to the formulation of this concept [21]. Similar observations suggest that donor heterogeneity may be one of the reasons for varying treatment outcomes, rather than the ineffectiveness of FMT as a method itself [9].

Multicenter studies have shown that the efficacy of FMT can vary significantly among donors - average cure rates ranged from 26% to 82% [14]. Consequently, selection algorithms were developed to exclude donors with low efficacy (<60%) based on early clinical outcomes, which helped maintain higher treatment efficacy [14].

In more restrictive protocols, “super-donors” are defined based on high bacterial density ($>1.75\times10^{11}$/g of stool), the exclusion of the dysbiotic Bact2 enterotype, and low levels of potentially harmful taxa, such as Fusobacterium, Escherichia/Shigella, and Veillonella [15]. At

the same time, it has been suggested that greater diversity in the donor microbiota and high numbers of taxa with anti-inflammatory effects (e.g., Faecalibacterium prausnitzii) may contribute to better clinical outcomes [17].

However, these relationships are not entirely clear and require further validation, as the results vary across studies and patient cohorts.

Although the concept of a "super-donor" suggests the possibility of optimizing FMT by selecting material with a specific microbiological profile, current data indicate high variability in outcomes and the absence of universal biomarkers of efficacy [21]. Additionally, the effects of FMT may depend on the specific condition - different microbiota profiles are beneficial in rCDI, while others are beneficial in inflammatory bowel disease (IBD) or metabolic disorders [22].

In clinical practice, FMT remains a complex procedure involving the preparation of the material (mixing, filtration, cryopreservation) and various methods of administration, such as colonoscopy, oral capsules, or administration via gastrointestinal tubes [24]. Ultimately, it is emphasized that the donor's profile can significantly influence the therapeutic effect, although the mechanisms underlying this phenomenon are not yet fully understood.

Therefore, the term "super-donor" should currently be viewed more as a concept than as a clearly defined clinical term, requiring further validation studies and standardization of criteria for evaluating the donor's microbiota.

7. FMT Safety and Donor Selection

7.1 Risk of Pathogen Transmission

The safety of FMT is limited by the potential risk of transmitting pathogens along with fecal material. As mentioned in the literature, the short- and long-term safety of FMT has not yet been fully established, and side effects may include diarrhea, bloating, fever, and inflammatory bowel disease flare-ups [5,25].

The most serious risk is systemic infection. Cases of bacteremia caused by multidrug-resistant strains of Escherichia coli following FMT have been reported, including fatal cases, which underscores the importance of rigorous donor selection [2,19]. Consequently, donors are excluded if they have, among other things, HIV, HBV, HCV, syphilis, or HTLV infections, as well as in cases of current infections or high-risk behaviors [4].

Standard testing includes blood and stool tests for major viral, bacterial, and parasitic pathogens, including multidrug-resistant organisms (MRSA, VRE, ESBL) [2,4,13,25]. To

reduce risk, material is quarantined and donors are retested after the serological window period [13]. Despite these procedures, rare but severe complications, such as sepsis or toxic megacolon, continue to be reported in the literature [5].

7.2 Regulations

The safety of FMT is regulated by agencies such as the FDA, which has issued warnings regarding the risk of pathogen transmission and the need for expanded donor screening [12]. Recommendations include, among other things, additional testing for selected intestinal pathogens and multidrug-resistant bacteria following adverse events associated with FMT.

Furthermore, the need to follow infection-prevention standards during the preparation and administration of the product is emphasized [5]. In clinical practice, this means increasingly comprehensive protocols for the screening and monitoring of donors and biological materials.

7.3 The Importance of Rigorous Screening

The donor screening process is extremely selective - only a small percentage of candidates complete the full evaluation procedure [2]. The reason for such a strict process is the need to minimize the risk of pathogen transmission and ensure the safety of recipients.

Despite extensive screening, adverse events may still occur. According to systematic reviews, most of these events are mild and include gastrointestinal symptoms or temporary fever, while severe complications are rare and often related to the administration procedure itself [18].

At the same time, available data indicate that current screening protocols significantly reduce the incidence of serious adverse events, which confirms their high effectiveness in clinical practice [18].

8. Standardization and Stool Banks

The standardization of FMT procedures and the development of stool banks have significantly improved the availability and safety of the therapy. In clinical practice, donor material is typically prepared from approximately 30–50 g of stool, which is mixed with saline, then processed into capsules or a suspension, and stored in cryogenic conditions [5]. It has also been reported that 45–50 g of donated stool samples were processed and stored at -80°C until use [1].

A key aspect of FMT's development is the ability to use either fresh or frozen material. Studies indicate that frozen and fresh stool demonstrate the same efficacy and safety [2], and extended

storage at −80°C does not adversely affect the efficacy of the therapy [6]. This simplifies logistics and increases the availability of material, particularly in the stool bank model.

Sperm banks, such as OpenBiome, enable the centralization of the donation process and the standardization of procedures. At the same time, the selection process remains very rigorous - only about 2-5% of candidates pass the full qualification process, and in one large program, only 3% of more than 15,000 candidates were ultimately qualified [2,23]. Such a restrictive selection process is necessary to ensure the biosafety and quality of the material.

The development of stool banks also reduces the need to match a single donor to a single recipient, a process that was previously costly and time-consuming [2]. The modern approach allows for the use of multiple donors and samples, which increases the flexibility of therapy. At the same time, it has been noted that combining samples may carry risks and requires caution, although it may improve the functional profile of the microbiota [9].

In recent years, the importance of washed microbiota transplantation (WMT) has been growing; through repeated centrifugation and filtration, it can improve the safety profile compared to conventional FMT [3]. At the same time, various routes of administration are being developed - ranging from oral capsules to enemas to colonoscopic administration - all of which demonstrate comparable efficacy, with the route to the large intestine being the most commonly preferred [5,26].

The main advantages of standardization include the repeatability of procedures and improved safety, while the limitations include costs, logistics, and a limited number of qualified donors.

9. Issues and Controversies

One of the main limitations of FMT is the lack of an international consensus on how to assess the quality of donors and the material, which makes it difficult to standardize and compare study results.

The concept of a “superdonor” is also controversial. Although it is associated with better FMT outcomes in inflammatory bowel disease, this does not apply to rCDI, where the donor’s profile does not affect clinical efficacy [2]. This suggests that the importance of the donor depends on the specific disease.

The criteria for excluding certain groups, such as healthcare workers, also remain controversial [7]. Despite the theoretical risk of pathogen colonization, the available data do not confirm a significantly higher incidence of such cases [4].

Another limitation is that most studies focus on rCDI [5], which limits the ability to generalize the findings to other FMT indications, such as metabolic diseases or neuropsychiatric disorders [27].

Differences between populations further complicate standardization. It has been pointed out that European and American guidelines do not always take into account differences in diet, BMI, and the microbiome among Asian populations; for this reason, more complex donor eligibility systems have been developed in China [19].

Other key issues include the lack of standardized protocols and regulatory differences between countries, which raises the important question of whether every healthy donor can be considered an equally suitable source of material for FMT.

10. The Future of Donor Selection

The future of FMT donor selection is moving toward greater precision, standardization, and personalized therapy. It has been suggested, among other things, that a 12-month antibiotic-free period prior to transplantation is optimal, but this finding requires confirmation and further research, suggesting that eligibility criteria may undergo further changes as new data become available [1].

The design of microbiome-focused studies is considered increasingly important. As highlighted, the rational selection of donors during the planning stage and the use of a priori hypotheses and retrospective analyses may increase the efficacy of FMT, particularly in diseases where the response depends on the composition of the microbiota [9]. At the same time, it has been suggested that a multi-donor approach may reduce the risk of failure associated with an incompatible microbiota profile, although the single-donor model remains important for identifying mechanisms of efficacy and complications control [15].

Another important area of development is the customisation of therapy within the donor–recipient model. Data indicate that the efficacy of FMT depends not only on donor characteristics but also on recipient factors, such as genetics, immunity, diet, and antibiotic exposure, which influence the colonization and persistence of the transplanted microbiota [22]. This suggests the need to consider the patient's individual sensitivity when selecting the transplant material.

In the future, donor selection may be supported by artificial intelligence tools and predictive microbiome models. Another option being considered is moving away from traditional FMT in favor of more controlled approaches, such as postbiotic preparations, which may offer greater predictability and safety in therapy.

11. Conclusions

Donor selection is one of the key factors determining the effectiveness of fecal microbiota transplantation, as the quality and composition of the donor's microbiota can influence the clinical outcome of the therapy and the durability of colonization in the recipient. Data from the literature indicate that differences among donors can lead to significant variations in treatment outcomes, underscoring the importance of careful screening and control of biological material.

At the same time, the current approach to donor selection remains inconsistent, and screening criteria vary across centers and countries. Consequently, there is a clear need for further standardization of procedures, both in terms of laboratory testing and the clinical and microbiological evaluation of donors. Standardizing protocols could improve safety and the comparability of test results.

In the future, the development of FMT will most likely move toward precision medicine, taking into account the individual characteristics of the donor and recipient, as well as more advanced methods of microbiome analysis. The integration of clinical, microbiological, and bioinformatics data may enable a more effective and personalized selection of therapies.

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Author's contribution:

Conceptualization, Karolina Grabowska, Patrycja Kardacka

Methodology, Patrycja Kardacka

Software, Karolina Grabowska

Check, Karolina Grabowska

Formal analysis, Patrycja Kardacka

Investigation, Karolina Grabowska

Resources, Patrycja Kardacka

Data curation, Karolina Grabowska

Writing - rough preparation, Karolina Grabowska, Patrycja Kardacka

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