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Original article

Donor screening for fecal microbiota transplantation with a direct stool testing-based strategy: a prospective cohort study

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ABSTRACT

Fecal microbiota transplantation (FMT) is effective against recurrent *Clostridioides difficile* infection (rCDI), but its safety is jeopardized by the potential transmission of pathogens, so international guidelines recommend either a quarantine or a direct stool testing. Whereas reports of the quarantine-based approach are emerging, data on the direct testing-based approach are not available. Our aim is to report outcomes of a donor screening framework for FMT including direct stool testing.

In this prospective cohort study, all donor candidates recruited at our FMT centre underwent a four-step screening process to be enrolled as actual donors. Each collected stool donation was then evaluated with a direct stool testing including a molecular assay for gut pathogens and a culture assay for multi-drug resistant organisms (MDRO).

From January 2019 to June 2023, 72 of 227 candidates (32%) were considered eligible and provided 277 stool donations. Ninety-nine donations (36%) were discarded for positivity to intestinal pathogens, most commonly enteropathogenic *Escherichia coli* (n = 37) and *Blastocystis hominis* (n = 20). Overall, 337 stool aliquots were obtained from 165 approved donations. All suspensions were used for patients with rCDI, and no serious adverse events or clinically evident infections were observed at 12 weeks after procedures.

In our study, screening of donor faeces including direct stool testing led to the discard of a considerable rate of stool donations but was also extremely safe. This approach may represent a reliable strategy to guarantee the safety of FMT programs, especially in countries with high prevalence of MDRO.

1. Introduction

Fecal microbiota transplantation (FMT) is a highly effective treatment for recurrent *Clostridioides difficile* infection (rCDI), with cure rates of nearly 90% [1], and is recommended by international guidelines for the management of this disorder [2]. Despite its high efficacy, FMT is still considerably underdiffused, as only few centers provide it across Europe, with an estimated coverage of nearly 10% of the current need [3]. Several factors prevent the wide diffusion of FMT, including bureaucracy issues, lack of expertise, and perceived safety risks.

In recent years, stool banks have arisen worldwide both to satisfy the increasing clinical request for FMT and to guarantee high levels of quality standards, proving effective in providing FMT at extremely safe conditions [4–6].

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However, the transmission of multi-drug resistant organisms (MDRO) has been recently identified by the USA Food and Drug Administration (FDA) as a potential risk related to FMT [7]. This alert was spread after the report of two immunocompromised patients who developed bloodstream infection, which was fatal in one of the two cases, from an extended-spectrum ß-lactamase-producing *Escherichia coli* strain received from a stool donor in the context of a FMT clinical trial where the presence of MDRO was not tested [8]. Later, the FDA was also notified of the suspected transmission of enteropathogenic *E. coli* (EPEC) in two patients, and of Shigatoxin-producing *E. coli* (STEC) in four patients, following FMT for rCDI [9]. After this further alert, the FDA required donor stool to be tested by nucleic acid amplification tests (NAAT) for EPEC and STEC [10].

Current FMT guidelines suggest several approaches to avoid the transmission of pathogens, as they recommend either a quarantine of donated feces or a direct testing with a rapid molecular assay for gut pathogens to be assessed in every stool donation [4,5].

Emerging evidence, coming from a retrospective report of 66 FMT donors, has proven the safety of periodic donor screening with a quarantine period [11]. Currently, data of a direct stool testing-based approach are still not available. Our aim is to report outcomes of a donor screening framework for FMT including direct stool testing.

2. Methods

2.1. Study design

In this single-centre, prospective, observational cohort study, we included all donor candidates recruited at the FMT centre of the "Fondazione Policlinico Universitario A. Gemelli IRCCS" (Rome, Italy) from January 2019 to June 2023, as part of our FMT program for rCDI in clinical practice.

All donor candidates underwent a four-step screening process to be enrolled as actual donors. Every single stool donation, collected from actual donors, was then evaluated with direct stool testing, which includes a molecular assay for gut pathogens and a culture assay for multi-drug resistant organisms (MDRO). Fig. 1 shows the complete screening process.

We recorded the number of discarded candidates as well as the specific step of the screening process where they have been excluded, and the reason for exclusion. We also recorded the pathogenic strains identified at the direct stool testing. If donors were asymptomatically positive for pathogens at the direct stool testing were temporarily excluded and re-tested after 4 weeks.

All approved stool donations were manipulated, frozen, and stored at −80 °C for further use. They were finally thawed and used for FMT when needed. A small (4 g) stool sample from each aliquot was stored at −80 °C for further analyses in case of post-FMT adverse events.

2.2. General framework of the donor screening process

The framework of our FMT donor screening included firstly an evaluation of donor candidates and then, if they were considered eligible and became actual donors, direct testing of stool donations.

Donors were selected following the international guidelines on FMT and stool biobanking [4,5] as well as the indications from the Italian National Transplant Centre [12]. Our framework was continuously updated over time, with a timely alignment with the most recent international recommendations concerning safety issues associated with COVID-19 and Monkeypox virus [13,14].

Donors were recruited both among young healthy volunteers and patients' relatives, who were motivated to become universal donors. We preferred to recruit donors living close to our FMT centre to minimise the risk of drop-out. Due to the known correlation between ageing and gut microbiota imbalance [15], donors <50 years old were preferred, but healthy volunteers up to 60-year-old were considered eligible if they had undergone a timely colorectal cancer screening, as recommended by international guidelines [4,5].

2.3. Screening of donor candidates

Donor candidates were screened through a four-step process. The first step involved a comprehensive questionnaire, detailed in Table 1, aimed to assess the general health status of the candidate and rule out known infectious disorders, risk factors for infectious disorders, or conditions that can potentially impair gut microbiota.

If the questionnaire did not reveal any critical issue, donor candidates progressed to the second step, that included a panel of blood and stool exams to exclude transmissible pathogens, as listed in Table 2.

Donors who passed the laboratory screening were then convened to our centre for stool donation. The third and the fourth

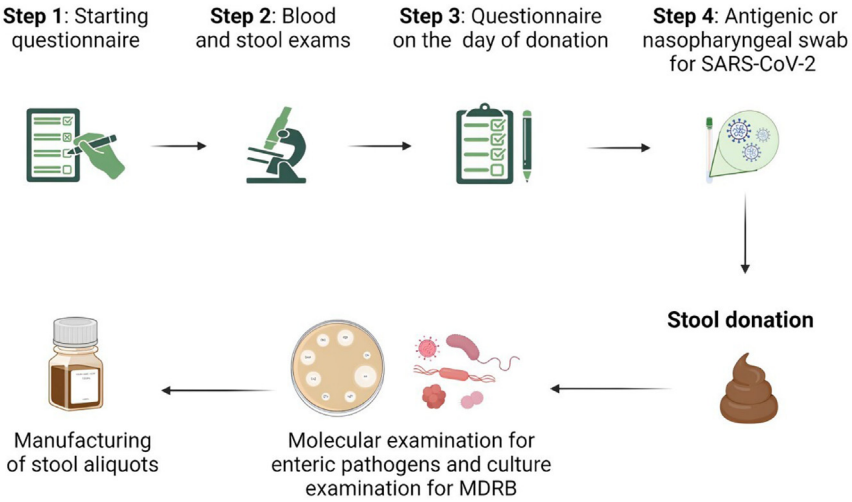

Fig. 1. Overview of the whole screening process.

Table 1
Preliminary questionnaire for donor screening.

Starting questionnaire
Drugs that can alter gut microbiota
Use in the last three months of:
- systemic antimicrobial drugs
- immunosuppressant agents
- chemotherapy
- daily use for over three months of proton pump inhibitors
Disorders potentially associated with the disruption of gut microbiota
- Personal history of chronic gastrointestinal disease, including functional gastrointestinal disorders; inflammatory bowel disease; coeliac disease; other chronic gastroenterological diseases or recent abnormal gastrointestinal symptoms (eg, diarrhea, haematochezia, etc.)
- Personal history of cancer, including gastrointestinal cancers or polyposis syndrome, and First-degree family history of premature colon cancer
- Personal history of systemic autoimmune disorders
- Obesity (body mass index >30) and/or metabolic syndrome/diabetes
- Personal history of neurological/neurodegenerative disorders
- Personal history of psychiatric/neurodevelopmental conditions
Know history or risk behaviors for infectious disease
- History of HIV, hepatitis B or C viruses, syphilis, human T-lymphotropic virus I and II
- History of MPXV in the previous 21 day/occurrence of signs and symptoms suggestive of MPXV infection in the previous 21 days/Close contact with people who are infected or suspected of being infected with MPVX in the previous 21 day/Recent travel to areas affected by home-grown confirmed cases of MPX or MPX-endemic countries
- Current systemic infection
- Use of illegal drugs
- High-risk sexual behavior
- Previous tissue/organ transplant
- Recent hospitalization or discharge from long-term care facilities
- High-risk travel/engaged in medical tourism

step of the screening were repeated at each donation. The third step involved the completion of an additional questionnaire to rule out the occurrence of risky situations (i.e. anonymous sexual contacts or use of drugs), any systemic or gastrointestinal symptoms, and/or the use of antibiotics, since the first questionnaire. Donors who passed this further questionnaire underwent, as fourth step of the screening, a nasopharyngeal swab to rule out the presence of SARS-CoV-2 infection [13].

If the donor candidate successfully completed all these steps, her/his faeces were accepted for evaluation and were brought to the microbiology laboratory for further exams. Donors could collect feces at home or at our centre, in a dedicated toilet. They received a disposable plastic bag to be opened over the toilet for faeces collection. Those collecting feces at home were asked to deliver stool in a cooler box and an ice pack immediately after defecation, to assure their manufacturing within 6 h, as recommended by international guidelines [4,5].

Table 2
Blood and stool exams for donor screening.

Blood exams
- Complete blood cell count
- Liver enzyme (Aminotransferases)
- Bilirubin
- Creatinine
- C-reactive protein
- Serology for Hepatitis virus (HAV, HBV, HCV, HEV) and Human immunodeficiency virus (HIV)
- Nematodes (*Strongyloides stercoralis*)
Stool exams
- *Clostridioides difficile* (GDH, Toxin A/B Enzyme Immunoassay)
- Microscope and molecular examination for Helminths and pathogenic Protozoa as *Entamoeba histolytica*, *Blastocystis hominis*, *Dientamoeba fragilis Giardia lamblia*, *Cryptosporidium* spp, *Cystosospora belli*, *Cyclospora cayetanensis* and Microsporidia
- *Helicobacter pylori* fecal antigen

All donors repeated clinical assessment and the whole set of laboratory testing every 8 weeks.

2.4. Direct stool testing

All stool donations collected from qualified donors underwent direct stool testing immediately after their arrival in the microbiology laboratory. The direct stool testing included both a rapid molecular assay for common intestinal pathogens and a culture assay for detection of MDRO.

2.4.1. Rapid molecular assay for intestinal pathogens

Nucleic acid extraction was performed by QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany), using at least 200 μg of feces.

Real-time PCR (RT-PCR) analyses were carried out to declare fecal donation "pathogen free" bypassing sensitivity limitations of conventional methods. Specifically, RT-PCR Allplex™ Gastrointestinal Panel Assays (Seegene, Seoul, Korea) for the comprehensive detection and identification of common gastrointestinal pathogens (i.e., including the following bacteria: Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shigatoxin-producing *E. coli* (STEC), *E. coli* O157, *Aeromonas* spp., *Campylobacter* spp., toxigenic *C. difficile*, *Salmonella* spp., *Shigella* spp./Enteroinvasive *E. coli* (EIEC), *Vibrio* spp., and *Yersinia enterocolitica*; the following viruses: Adenovirus, Astrovirus, Norovirus GI\GII, Rotavirus, and Sapovirus; and the following parasites: *Blastocystis hominis, Cryptosporidium* spp., *Cyclospora cayetanensis, Dientamoeba fragilis, Entamoeba histolytica*, and *Giardia lamblia*. Moreover, feces were tested for SARS-CoV-2 detection by Allplex™ SARS-CoV-2 kit (Seegene, Seoul, Korea) [16]. Results of this molecular testing were available in nearly 2 h from the analysis.

2.4.2. Stool culture for MDRO

Chromogenic agar media were used for the isolation of antibiotic resistant bacteria including vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum beta-lactamase bacteria (ESBL) and carbapenamase producing bacteria (CARB). The agar plates used in our protocol were manufactured by BioMérieux, Craponne-France (Chromid™Carba SMART, Chromid®VRE, Chromid®ESB, Chromid®MRSA SMART). Nearly 24–48 h were needed to obtain results of the culture.

2.5. Manipulation of donor feces and fecal microbiota transplantation

Immediately after the direct stool testing, feces were manipulated at the microbiology laboratory as previously described [17], following international guidelines [4,5], and stored at −80 °C. Feces were manufactured without waiting for testing results, to preserve anaerobic taxa. Each stool donation was divided, whenever possible, based on fecal weight, in ready-to-use aliquots of nearly 60 g, as previously described [17]. Each aliquot was labelled with a unique barcode of the corresponding donor, and with the date of collection/manipulation, to assure its complete traceability, as recommended by international guidelines [4,5].

Stool donations <50 g were deemed insufficient to prepare a stool aliquot and were discarded. All manufactured aliquots were quarantined for 24–48 h while waiting for results of the direct stool testing, and positive donations were then discarded.

Each approved fecal aliquot was thawed at room temperature the day of the scheduled FMT. All fecal infusions were performed as already described, and adverse events were recorded for up to 12 weeks after FMT [17,18].

3. Results

3.1. Overall results

From January 2019 to June 2023 we screened 227 donor candidates, of which 72 (32%) were finally enrolled (39 females, 53%; mean age 37 years old, range 22–60), with consequent overall collection of 277 stool donations. Only 165 of them (59.6%) were found to be eligible, while 99 (35.7%) were excluded at the direct stool testing and 13 (4.7%) were discarded for insufficient amount of faeces (<50 g). Finally, 337 FMT aliquots were obtained from these eligible donations.

3.2. Donor testing

The complete flow chart of donor testing is summarized in Fig. 2. Overall, 227 donor candidates were assessed for eligibility between January 2019 and June 2023. Of them, 113 (50%) declined for logistic reasons (lack of time, travelling costs and difficulties, being uncomfortable at donating feces). Of the 114 remaining candidates, five (4%) were excluded at the donor questionnaire and eight after blood and stool testing (7%). Specific reasons for exclusions are described in Fig. 2. Twenty-six qualified stool donors declined to donate after blood and stool screening (24%), another one (0.4%) was excluded for antibiotic intake reported in the questionnaire submitted the day of donation, and two donors (0.8%) were rejected for positivity of the nasopharyngeal swab for SARS-CoV-2. Finally, 72 candidates qualified as stool donors (32%). On average, these donors repeated the screening process twice, ensuring their availability for sixteen weeks on average (range 8–112 weeks).

3.3. Direct stool testing

The whole flow chart of direct stool testing is summarized in Fig. 3, while Fig. 4 offers a detailed overview of pathogens positivity in our cohort. Overall, 99 (36%) of 277 stool donations were excluded at the direct stool testing for detection of one or more pathogens and/or MDRO.

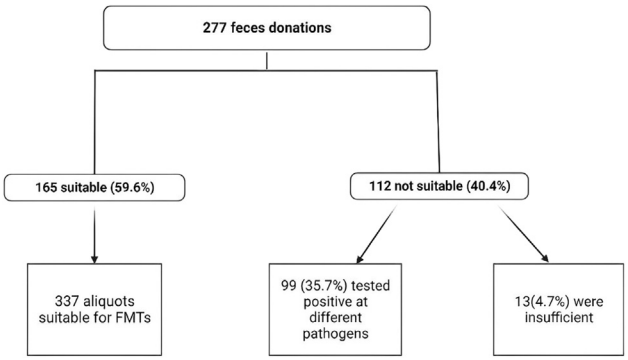

Fig. 3. Flow-chart of the direct stool testing.

Gram negative bacterial pathogens were present in 82 (29.6%) of 277 stool donations, and the *E. coli* was the most commonly identified species, being found in 64 samples (23.1%). EPEC was the most prevalent strain, with 37 positive samples (13.4%).

Parasites were identified in 24 donations (8.7%), mainly *B. hominis* (n = 20, 7.2%). Gram positive pathogens were present in 7 cases (2.5%), particularly *C. difficile* (n = 5, 1.8%). Finally, viruses were the least commonly observed pathogens, with 5 cases (1.8%), of which two belonged to Norovirus (0.7%).

Seventeen donations (6%) were excluded after the culture assay for the presence of ESBL-producing Enterobacteriaceae (*E. coli*, n = 16; *Klebsiella pneumoniae*, n = 1).

All positive donors reported no symptoms on the day of donation. Therefore, they were not treated clinically, and we observed spontaneous clearance of the pathogen at an average 4-week follow-up, after repetition of the direct testing.

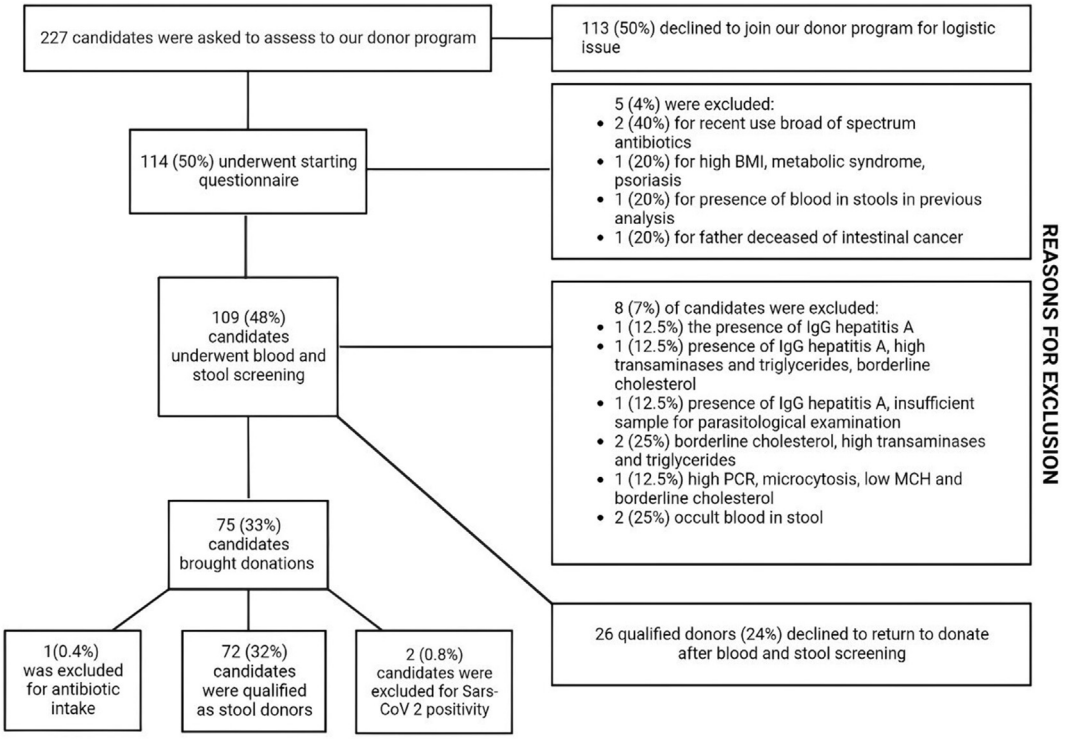

Fig. 2. Flow-chart of the donor selection process.

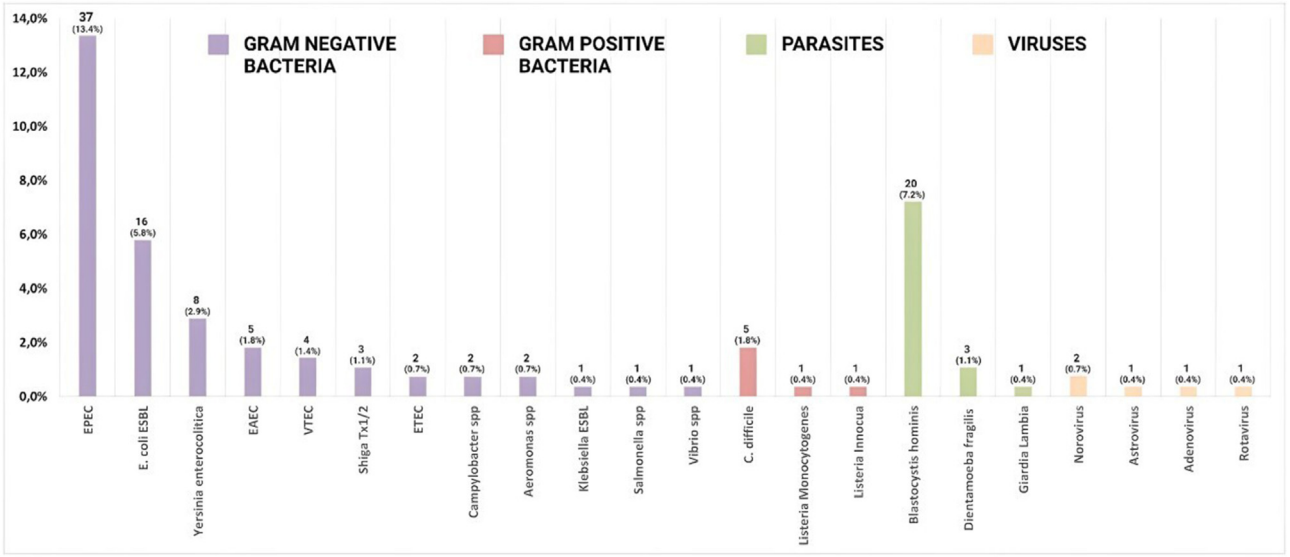

Fig. 4. Overview of detected intestinal pathogens in our cohort (color should be used for this figure in print).

Notably, we found a seasonal variation among positivities of donations, as most pathogens were observed in September (17/87 = 19.5%, calculated from 2019 to 2022).

As shown in Fig. 5, we observed an upward trend in the number of donors and stool donations from 2019 to 2023.

Overall, 337 fecal aliquots were obtained from the 165 suitable stool donations, The mean percentage of validated donations per donor was 47%. The mean number of validated donations was 3.75 (range 1–30). All suitable donations were used for FMT. At 12-week follow-up, we did not observe any serious adverse event, nor clinical manifestations of infection, in FMT recipients.

4. Discussion

In our 4-year prospective study we reported on the outcomes of a donor screening framework for FMT which included direct stool testing. We screened a total of 227 donor candidates, of which only 72 (32%) qualified as actual stool donors. These donors contributed a total of 277 stool donations, of which 99 (36%) were excluded by direct stool testing.

Based on available data, rates of donor exclusion range widely among different stool biobanks, from 2% to 31% [19–22]. This variation could be attributed to differences in the pre-screening selection of donor candidates. In our study, 113 (50%) individuals were not included in the screening program due to logistical issues such as distance from the hospital or inability to donate regularly due to workload. In another cohort with similar enrolment rates (31%), Costello et al. searched for donors among university students who were more likely to be healthier than older individuals and to donate repeatedly due to their proximity to the FMT center [19]. These data suggest that the adoption of pre-selection strategies may reduce the rates of donor discard during the actual screening.

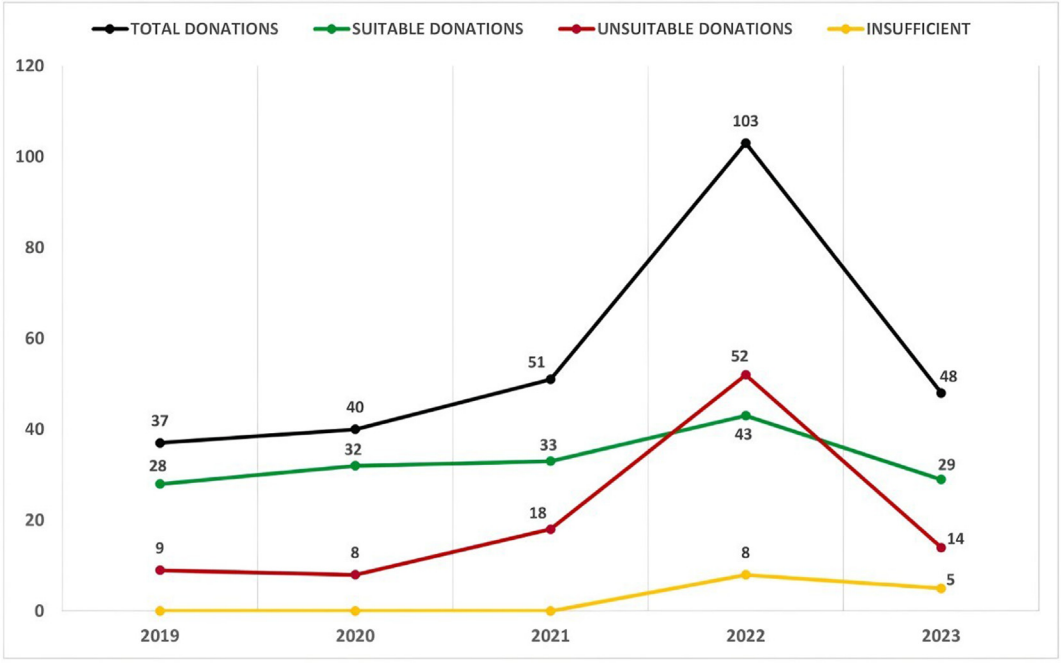

Fig. 5. Trends in the number of donors and stool donations throughout the study period.

As donor screening is a time-consuming and costly process, the identification of strategies that can predict the suitability of donor candidates in the pre-screening phase is crucial and deserves further research, although it may be difficult for the majority of hospitals, being suitable mostly in referral FMT centres.

In our cohort, nearly 36% of collected stool donations were excluded for positivity at the direct stool testing. EPEC was the most commonly identified bacterial pathogen, being found in nearly 13% of cases (n = 37 samples). These findings are consistent with previous studies that reported a significant presence of EPEC in asymptomatic carriers [23–25].

The second most relevant cause of positivity was *B. hominis*, which caused the exclusion of 20 (7%) donations. This finding falls within rates observed in previous cohorts, as Terveer et al. found it in 2% [21], and Kassam et al. in 13% [22], respectively, of asymptomatic donor candidates. Although the absolute number of positivities is not critical, this finding is relevant for several reasons. First, increasing evidence suggests that *B. hominis* is commonly found in healthy individuals, and its pathogenicity is now under debate [26]. Moreover, the Netherlands Donor Feces Bank (NDFB) Study Group has recently reported that the transmission of *B. hominis* from stool donors to recipients with rCDI did not result in any gastrointestinal adverse event nor had any impact on FMT efficacy [27]. Further and more consolidated evidence is needed to clarify if *B. hominis* should be kept as an exclusion criteria within the donor screening for FMT.

We also observed that most positivities occurred in September, which follows the preferred vacation period in Italy (July and August). As traveling abroad increases the risk of asymptomatic pathogens carriage, this finding may be associated with more frequent travels during this time [25–28].

Interestingly, we excluded only 7% of candidates at the stool and blood exams, and none of them for the presence of pathogens at standard stool testing. This finding can be explained by two main reasons. First, we invite only healthy subjects to participate our donor program. Moreover, all candidates underwent a thorough questionnaire to exclude those with gastrointestinal symptoms or other clinical or epidemiological risk factors for infectious gastroenteritis.

Additionally, we found that only five donations were discarded for the presence of viruses at the direct stool testing. The asymptomatic carriage of enteric viruses is rare in the general population and is usually higher in rural and non-modernized areas [29], being also more common in children than in adults [30]. Our results can be then explained as we recruited only adult donors from urban areas.

Despite the high cost and demand for our strategy, it ensured a high level of quality and safety for our FMT program. We observed no serious adverse events or clinically evident infections in our FMT recipients. Our results are especially pertinent given the higher prevalence of MDRO in Italy than in other European countries, and we identified ESBL-producing bacteria in 6% of our cohort (n = 17 samples) [31].

Recently, the NDFB Study Group demonstrated the effectiveness of quarantining donor feces to prevent the spread of MDRO in FMT recipients. However, the authors acknowledged that their study was conducted in a country with low endemicity for MDRO [32]. To the best of our knowledge, studies comparing these approaches in regions with varying prevalence of MDRO have not yet been conducted.

Strengths of our study are our innovative approach for donor screening, that encompasses direct stool testing including both a molecular assay for gut pathogens and cultures for MDRO, as well as its application in a large cohort of stool donors. However, our study has also some limitations. First, the study was carried out in a single centre, therefore our findings may not be generalised to other stool biobanks. Moreover, as we discarded all stool donations with pathogens, our study was not designed to assess the actual clinical risk of pathogens transmission through FMT, although a previous report shows that they, especially the MDRO, can be highly detrimental [8].

In conclusion, in this single-centre prospective cohort study we evaluated the outcomes of a FMT donor screening framework including direct stool testing.

Our approach led to the discard of a considerable rate of stool donations (36%), but also guaranteed a high safety level of our FMT program, as neither serious adverse events nor clinically evident infections were observed in FMT recipients (n = 337 procedures within the study period). Another advantage of our approach relies in the rapid (up to 48 h) availability of approved aliquots for clinical use (FMT). A donor screening framework including direct stool testing may represent a reliable alternative to the quarantine-based strategy, especially in countries with higher prevalence of MDRO. Further studies, designed to compare these approaches in different settings, are advocated in order to standardize the framework of FMT donor screening.

Ethics

All enrolled donors provided written informed consent. The study protocol was approved by the local ethics committee (0020270/18).

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CRediT authorship contribution statement

Debora Rondinella: Data curation, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Gianluca Quaranta:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Tommaso Rozera:** Data curation, Formal analysis, Methodology, Writing – original draft, Visualization. **Pasquale Dargenio:** Data curation, Visualization. **Giovanni Fancello:** Data curation, Investigation. **Irene Venturini:** Data curation, Investigation. **Alessandra Guarnaccia:** Data curation. **Serena Porcari:** Investigation. **Stefano Bibbò:** Investigation. **Maurizio Sanguinetti:** Supervision, Validation. **Antonio Gasbarrini:** Supervision, Validation. **Luca Masucci:** Supervision, Validation. **Giovanni Cammarota:** Supervision, Validation. **Gianluca Ianiro:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

AG reports personal fees for consultancy for Eisai S.r.l., 3PSolutions, Real Time Meeting, Fondazione Istituto Danone, Sinergie S.r.l. Board MRGE and Sanofi S.p.A; personal fees for acting as a speaker for Takeda S.p.A, AbbVie and Sandoz S.p.A; and personal fees for

acting on advisory boards for VSL3 and Eisai. GI has received personal fees for acting as speaker for Alfa Sigma, Biocodex, Illumina, Malesci, Sofar and Tillotts Pharma, and for acting as consultant/advisor for Biocodex, Malesci, and Tillots Pharma. The other authors have no potential competing interest to disclose.

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