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The Italian National Faecal Microbiota Transplantation Program: a coordinated effort against *Clostridioides difficile* infection

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Abstract
Clostridioides (previously *Clostridium*) *difficile* infection (CDI) is a common cause of antibiotic-associated diarrhea, whose symptoms range from mild diarrhea to life-threatening pseudomembranous colitis. CDI is characterized by significant recurrence rate following initial resolution and recurrent *C. difficile* infection (rCDI) represents an onerous burden for the healthcare systems. Conventional antibiotic-based approaches are generally used for the treatment of rCDI but the effective therapy remains elusive. Recently, the faecal microbiota transplantation (FMT) has emerged as an alternative therapeutic strategy against rCDI, with high treatment success rate. In 2018, the Italian National FMT Program was launched, with the aim to provide high quality standards in FMT application to adults with rCDI not responding to antibiotic therapy. Here, we sketch out the key characteristics and the progress of the Italian National FMT Program during the COVID-19 pandemic.

Key words
- faecal microbiota transplantation
- *C. difficile* infection
- antibiotic resistance
- COVID-19
- quality assurance

CLOSTRIDIOIDES DIFFICILE INFECTION AND FAECAL MICROBIOTA TRANSPLANTATION

The faecal microbiota transplantation (FMT) consists in transferring of a faecal sample from a healthy donor into the gastrointestinal tract of a patient, in order to modulate the gut microbiota. The gut microbiota includes bacteria, archaea and eukarya colonizing the gastrointestinal tract, and its composition is shaped by environmental factors, such as diet, and also by host immune system and genetics [1]. In terms of susceptibility to infectious diseases, the microbiota imbalance (dysbiosis) has a remarkable impact on the colonization resistance, that is the resistance provided by the microbiota against enteric pathogens [2]. Antibiotic therapy is a common cause of dysbiosis reducing bacterial diversity and abundance, and is considered a risk factor for the host infection by different pathogenic microorganisms enriched following the microbiota perturbation. A well-known example is *Clostridioides difficile* infection (CDI) characterized primarily by diffuse diarrhea and marked by a significant rate of relapse following antibiotic treatment [3-4]. Antibiotics deplete gut microbiota resulting in decreased microbiota signaling and diminished local and systemic immune responses to CDI [2]. The clinical symptoms of CDI range from mild diarrhea to fulminant pseudomembranous colitis associated with toxic megacolon, colonic perforation and multiorgan failure [4]. In 2017, there were an estimated 223,900 CDI cases in hospitalized patients and 12,800 deaths in the United States [5]; in 2016, CDI surveillance in Europe reported a total of 7711 cases, 5756 of which (74.6%) were healthcare-associated and 611/7711 (7.9%) cases were classified as recurrent infections (rCDI) [6]. Besides the substantial effect on patient quality of life, the management of rCDI, defined as a relapse of CDI symptoms within 2-8 weeks of successful treatment of the initial episode [7], is also a

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relevant burden for the healthcare systems; it has been reported in fact that the risk of developing a first rCDI is 25%, with a 40% probability of a second recurrence episode and an increased rate of re-hospitalization [8]. Conventional antibiotic-based approaches are generally used for the treatment of rCDI but the effective therapy remains a challenge. The antibiotic treatment options for patients include standard course or tapered and pulsed regimen of vancomycin, vancomycin followed by rifaximin or a standard course of fidaxomicin [7]. Recently, FMT has emerged as an alternative and viable strategy against rCDI and both the European Society for Microbiology and Infectious Diseases and the American College of Gastroenterology recommended FMT for patients with multiple recurrences of CDI who failed therapy with the appropriate antibiotic agents [9, 10]. High treatment success rates have been reported and FMT has been well tolerated by patients with few reports of adverse events [10].

Considering that the role of FMT in clinical practice is evolving, it is relevant to establish standardized procedures that meet specific requirements of quality, safety and efficacy. In 2017, the European Consensus guidance document defined indications and methodology for the use of FMT in CDI treatment [11] and, in 2019 an international consensus conference on stool banking for FMT has been published with the aim to provide a guidance on the general organization of a stool bank including issues for donor recruitment/screening, preparation and storage of faeces as well as for release faecal suspensions to clinical centers [12]. It is worth mentioning that a common approach for the faecal microbiota regulation does not exist in Europe and Member States are free to decide on the most suitable framework either by establishing a specific regulatory framework at national level or by applying one of the existing legislative frameworks, such as national requirements for tissue and cell transplantation [13]. However, many European countries have no regulation on FMT at all; globally, the faecal microbiota classification is a demanding task because the underlying mechanism of action and the active component are still to be completely understood and FMT therapy does not fit entirely in any regulatory framework. In Italy, the faecal microbiota has been classified as human cell/tissue product and regulated according to DL 191/2007 and DL 16/2010, in compliance with Directives 2006/17/EC and 2006/86/EC implementing Directive 2004/23/EC on the quality and safety of tissues and cells [14, 15]; currently, the FMT regulatory application is falling within the remit of Italian National Transplant Center (CNT), which was appointed as the coordinator of the Italian National FMT Program launched in 2018 by the Italian Ministry of Health.

THE ITALIAN NATIONAL FMT PROGRAM: KEY CHARACTERISTICS AND STATE OF THE ART

The Italian National FMT Program was established with the aim to provide high quality standards in FMT application to adults with rCDI not responding to antibiotic therapy, ensuring best clinical practice for the patient care. The FMT Program was addressed to all public health structures and Regional hospitals, placed on the national territory, with appropriate expertise and facilities, including a gastroenterology unit, endoscopy service, clinical ward and outpatient clinic, a processing laboratory (biosafety level 2), as well as a microbiology testing laboratory and either an infectious diseases service or expert advice.

The FMT Program document provides a technical and operational guidance on how to set up the FMT process and CNT is in charge of evaluating if clinical centers fulfill the advised criteria needed for implementing a FMT service. Among the others, the guidance includes specific requirements relating to donor selection, a crucial issue to assure a safe FMT treatment. In particular, in agreement with the recommendations of the European FMT Working group [11], both related and unrelated donors may be enrolled and the donor selection/recruitment consists of three different steps: 1) a written medical interview to exclude history and risk factors; 2) blood and stool testing at most 4 weeks before donation to check the donor for any potentially transmittable disease; 3) questionnaire and stool testing on the day of donation. The screening panel for donor stool testing was established with the purpose to detect not only common enteric pathogens and faecal parasites but also multi drug-resistant organisms, including meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci, extended-spectrum β-lactamase producing and carbapenemase-producing Enterobacteriaceae, in order to avoid the transmission of microorganisms that could lead to serious or life-threatening infections as it has already been reported [16]. Noteworthy, due to the ongoing COVID-19 pandemic the standard donor screening protocol was strengthened with additional measures to minimize the risk of COVID-19 infection [17]. In particular, according to the indications from an international FMT expert panel [18], CNT recommended that potential donors should be asked for: known diagnosis of laboratory confirmed SARS-CoV-2 infection; appearance of specific symptoms associated to COVID-19 including fever, cough, fatigue, muscle pain, dyspnea and headache; close exposure to subjects with suspected or proven infection, within the previous 30 days. Moreover, RT-PCR assay for SARS-CoV-2 on nasopharyngeal swab specimen and stool sample was strongly advocated, in accordance with the opinion of worldwide FMT experts [18, 19]. Recently, CNT indications for the screening of stool donors vaccinated for SARS-CoV-2 have been released [20].

Regarding the stool handling, another critical step of FMT procedure, laboratories are required to use standardized analytical techniques and the protocol for the preparation of both fresh and frozen faecal material is fully detailed in the Italian FMT guidance. Currently, the use of frozen faecal material is the preferred option to reduce the potential risk of SARS-CoV-2 transmission associated with FMT, since the freezing allows to quarantine the stool sample until screening results are available. Overall, the participants to the Program are required to adhere to standard operating procedures for the processing and to apply qualitative and quantitative

quality-control tests for the release of the final product to be delivered to the patient (via colonoscopy or retention enema or alternatively into the upper gastrointestinal tract). Finally, in order to ensure the traceability of the entire FMT process from the donor to the recipient, the product flow data need to be recorded and appropriate documentation relating to each step of the procedure must be prepared. In this respect, it is worth mentioning that the development and the implementation of the quality management and full traceability systems are key elements taken into account by CNT during the authorization process, which consists of both documentation review and on-site inspections.

To date, 18 health structures requested for the participation in the Italian National FMT Program, 11 from the Northern, 6 from the Middle and 1 from the Southern Italy, with an evident decreasing North-to-South gradient. Concerning the assessment of the minimum requirements, only 9 out of 18 FMT centers were audited, as the other applicants submitted incomplete or inadequate documentation in order to perform on site inspections. Reference guidelines and suggestions on how to deal with relevant issues are provided to the centers, with the aim to improve documentation and facilitate the implementation of corrective actions to overcome any critical deficiencies.

Out of the 9 inspected centers, 4 passed successfully each of the evaluation steps and were authorized to participate in the National Program, whereas 5 are still implementing the appropriate post-inspections corrective measures. It should be noted that the COVID-19 outbreak can certainly explain the slow progress in implementing the Program: approximately two thirds of the applicants come from Italian regions primarily impacted by the coronavirus spread (Lombardia, Piemonte, Emilia-Romagna, Liguria, Toscana) and, in addition, the clinical microbiology laboratories have undergone a progressive adaptation to meet growing demand for SARS-CoV-2 testing, with a consequent considerable reduction of all non-COVID-19 related testing activities. Furthermore, hospitals interested in participating in this Program are now playing a crucial role as COVID-19 vaccine hubs, and they are very committed to this task.

Currently, the FMT center within Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome is the only one to carry out microbiota transplants, as the service has been able to adapt its operational workflow during COVID-19 pandemic, in order to continue offering FMT treatment to patients with rCDI [21]. With regard to the surveillance of FMT safety and efficacy, the CNT has developed a client-server application (MySQL database) to register the procedures carried out within the Italian National FMT Program and the users can easily enter data relating to donor, patient, stool sample processing, route of delivery, and transplant outcomes including any serious adverse events and/or reactions occurring during the follow-up period (8 weeks). This system ensures transplants traceability and represents the nationwide collection of faecal microbiota transplants. The main characteristics of all data collected prospectively from June 2020 up to March 2021 are shown in *Table 1*. In particular, 31 patients (male=17, female=14) were transplanted with frozen faeces from 6 donors (male=2, female=4; unrelated=5, related=1). The median age was 70 and 50 for the patients and donors, respectively, and stool samples from the same donor served, on average, 6 patients. At 31 March 2021, 24 patients completed the follow up with a transplant success rate of 100%, while the follow up is ongoing in the remaining 7 patients. Noteworthy, no serious adverse events and/or reactions were notified.

Table 1
Characteristics of treated patients, donors, transplants and outcomes

	N
Patients	31
Male/female	17/14
Median age (min-max)	70 (20-94)
Vancomycin/fidaxomicin treatment pre-FMT	31/0
Donors	6
Male/female	2/4
Median age (min-max)	50 (37-60)
Unrelated/related	5/1
Transplants	
Frozen/fresh material	31/0
Colonoscopy/enema/nasogastric tube	31/0/0
Single/sequential infusions in patients with completed follow-up	18/6
Outcomes	
Follow-up at 8 weeks completed/ongoing	24/7
Successful/failed outcome	24/0
Serious adverse events/serious adverse reactions	0/0

CONCLUSIONS

The establishment of the Italian National FMT Program allowed to evaluate if the Italian centers comply with specific quality and safety standards for FMT application in rCDI. Furthermore, the Program provides a surveillance system for collecting and analyzing several information on FMT treatment, including the number of screened donors and performed transplants, stool manipulation, infusion procedure, outcomes and follow-up data, monitoring any deviations from the standard procedures. On the basis of data available at this stage, FMT approach results in a well-tolerated and efficacious treatment for adults with rCDI refractory to antibiotic therapy. On the other hand, as the sample is restricted, further data are needed to confirm these results and additional FMT centers should contribute to the progress of the Program. In order to encourage participation in the Italian National FMT Program, dissemination and training activities were carried out, such as webinars and CME (Continuing Medical Education) courses involving the whole regional transplant

network. Regrettably, the current pandemic has largely impacted on healthcare systems and imposed the reduction of medical procedures and other services COVID-19 unrelated. However, it needs to point out that Lewandowski *et al.* recently observed a significant higher incidence of CDI in hospitalized patients with COVID-19 [22], raising concerns about a potential cause-effect relationship between SARS-CoV-2 infection and CDI occurrence.

Overall, the Italian National FMT Program represents a structured model in order to standardize and harmonize the clinical FMT procedures nationwide, so guaranteeing patients access to safe, high-quality and effective service. Once a significant amount of data is achieved, the next step will aim to set up the minimum organizational, structural and technological requirements to develop a stool bank and FMT specific clinical pathways, in order to provide an equitable, timely and cost-effective access to FMT treatment, according to the national regulatory frameworks for human cell/tissue products [14, 15]. Finally, the Italian National FMT Program may serve as a template to implement additional joint activities for other FMT potential applications in non-CDI settings, including other gastrointestinal diseases as well as metabolic diseases, neuropsychiatric and immunologic disorders [23]. In this context, it should be noted that, with the exception of CDI, FMT approach is currently considered an experimental treatment and shouldn't be performed without CNT approval, in according to the national legislative framework for the experimental transplantation [24].

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings.

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