Stool processing speed and storage duration do not impact the clinical effectiveness of fecal microbiota transplantation

Jessica R. Allegretti, Ryan J. Elliott, Alim Ladha, Mary Njenga, Kurt Warren, Kelsey O’Brien, Shrish Budree, Majdi Osman, Monika Fischer, Colleen R. Kelly, and Zain Kassam

Introduction

Fecal microbiota transplantation (FMT) is a recommended therapy to prevent recurrent Clostridioides difficile infection (CDI). Stool banks have emerged to enable safe widespread access by providing pre-processed, rigorously screened material that can be stored locally until clinically required. It has been established that frozen stool is non-inferior to fresh stool with regards to clinical efficacy in recurrent CDI. However, it is unknown whether variation in the length of stool processing time or storage duration of banked material may compromise bacterial viability and reduce clinical effectiveness. In-vitro work suggests that changes in viability are modest, however, given that the mechanism of action of FMT remains largely unknown, clinical data is paramount to help inform stool banking processes. This study aims to assess the impact of FMT processing time and FMT storage time on clinical effectiveness in CDI.

Methods

Quality assurance data from a large stool bank (OpenBiome, Cambridge, USA) was collected on (1) processing time from stool passage to freezing (2) freezer storage time prior to FMT administration and (3) clinical effectiveness of the FMT. Data were collected consecutively from 257 healthcare facilities from January 22, 2014 to March 8, 2016 on FMTs performed for recurrent CDI.

Stool processing was defined as the number of minutes from time of passage of stool to time of unit being placed in freezer after processing. Donors’ donations were collected at home using a stool collection kit. After passing the stool, donors close the container’s lid and place it in a resealable plastic bag for secondary containment. A staff member received the sample at a collection facility labels the donation with a donor identification number and the time of passage as reported by the donor. Each donation is diluted and aliquoted (depending on the final FMT preparation) with a glycerol-saline solution (12.5% glycerol in 0.90% w/v NaCl in water), and fully homogenized. OpenBiome produces two liquid preparations, a 250 mL preparation diluted at a 10:1 ratio (i.e., approximately 23 g of stool) intended for delivery by colonoscopy, sigmoidoscopy, or enema delivery, and a 30 mL preparation diluted at a 5:2 ratio (i.e., approximately 9 g of stool) intended for delivery by esophagogastroduodenoscopy or naso-jejunal tube. It is then placed in a freezer at −80°C. Each FMT preparation is derived from a single donation; stools from different donors or donations are never mixed. Processing is accomplished with a laboratory paddle-based stomacher with a sterile single-use microfilter. The stool is processed aerobically.

CONTACT
Jessica R. Allegretti jallegretti@bwh.harvard.edu Division of Gastroenterology, Brigham and Women’s Hospital, Boston, MA 02115

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Freezer storage time was defined as the number of days from the date the unit was created and placed in the freezer at OpenBiome to the date the unit was shipped to the receiving site. Notably, each treatment is shipped on dry ice with a warm mark indicator to ensure no significant temperature excursions during transit. Sites are advised to store the material at either $-20^\circ\text{C}$ or $-80^\circ\text{C}$ until thawed. The following recommendations regarding thawing are provided with each sample: Units are recommended to be thawed using a $30^\circ\text{C}$ warm water bath. Alternative methods include thawing at room temperature and thawing in a refrigerator. Thaw times will vary by product. Once thawed, a treatment can sit at room temperature for up to 4 hours, or in a refrigerator for up to 8 hours.

The primary outcome was physician-reported clinical cure per standard of care follow-up, 8-weeks post-FMT. Descriptive statistics and logistic regression analysis were conducted.

Results

Complete clinical effectiveness data from the treating healthcare facilities was returned for 1,924 FMTs, which were included in this analysis. The overall clinical cure rate at 8 weeks post FMT from physician-reported data across all FMT delivery modalities was 83.8%. The mean processing time was 83 minutes (SD ± 27 minutes, range: 20–165 minutes) (Figure 1a), and the mean storage time before administration was 139 d (SD± 67 d, range 8–524 d) (Figure 1b). Using univariate logistic regression, processing time ($p = .48$) and storage time ($p = .34$) had no statically significant impact on clinical cure rates of recurrent CDI post-FMT at week 8. An a priori subgroup analysis of processing time intervals and storage duration did not have a statistically significant impact on recurrent CDI cure. Material processed less than 2 hours from passage yielded an 83.7% efficacy rate (N = 1794) compared to 85.4% (N = 130) for material processed after more than 2 hours. With regards to storage, material stored for <6 months at $-80^\circ\text{C}$ (83.8%, N = 1473) was comparable in effectiveness to material stored for 6–12 months at $-80^\circ\text{C}$ (83.8%, N = 439) and for >12 months at $-80^\circ\text{C}$ (83.3%, N = 12), suggesting that frozen storage duration does not significantly impact the rate of clinical cure, although the sample size is small for material older than 12 months.

Conclusion

To our knowledge, this is the first large-scale study of the impact of stool processing time and material storage duration on real-world clinical effectiveness of FMT for the treatment of recurrent CDI. These data support previous in-vitro studies that have guided current best-practices and indicate that within the range allowed by stool bank protocols, FMT is robust to variation in processing and storage duration. This data acknowledges that longer processing time may be acceptable, though systemic testing of longer processing times needs to be done to inform practical guidelines for stool banking in this emerging field.

There were several limitations to this study. These include that the primary outcome was physician

Figure 1. (a) FMT efficacy by product storage time (b) FMT efficacy by product freezer storage time.
report of clinical efficacy, and we do not have stool testing or engraftment measured. In addition, we do not have data on how long the samples were stored at the receiving sites prior to use. Given the standard for processing at the time was limited to approximately 2 hours, we have limited data on processing times significantly longer than that, and this requires further exploration to determine the clinical impact. Recent pre-clinical work has suggested that longer processing times did not have a meaningful impact on the bacterial communities and therefore consensus guidelines have recommended processing time within 6 hours may be appropriate. We also recognize that while all consecutive patients in which results were reported were included, there may be reporting bias. Sites were not mandated to report their outcomes, though highly encouraged. Therefore reporting of outcomes is limited by which sites provided reports of their outcomes to OpenBiome. In addition, at the time this data was collected, purity and potency testing had not been defined in this space and therefore not measured. We do feel that there are several strengths as well including the large size of the data set and the variety of sites in which data was collected.

As the field continues to expand, this data may help to inform clinicians local stool banks with regard to processing and storage processing.

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Disclosure of potential conflicts of interest

ZK and SB are employed by and has equity in Finch Therapeutics Group. JRA and MF consult for Finch Therapeutics. No other conflicts of interest to report for any authors relevant to the work presented in this manuscript.

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ORCID

Alim Ladha http://orcid.org/0000-0003-3945-7924
Shrish Budree http://orcid.org/0000-0001-8345-7548