for the selection of subsets of the collection for testing in gnotobiotic mice, the determination of which members are responsible for effecting the phenotype, and the elucidation of the mechanisms underlying these effects. The models used may inform the design and interpretation of clinical studies of the very individuals and populations whose microbiota are selected for creating these models.

Human-to-human fecal microbiota transplantation (FMT) is currently the most direct way to establish proof-of-concept for a causal role for the microbiota in disease pathogenesis. A human donor's feces are provided to a recipient via nasogastric tube or another technique. Numerous small trials have documented the effects of FMT from healthy donors to recipients with diseases ranging from *C. difficile* infection to Crohn's disease, ulcerative colitis, and type 2 diabetes. Only a few of these studies have used a double-blind, placebo-controlled design.

In a double-blind, controlled trial involving men 21–65 years old with a body mass index of >30 kg/m^2 and documented insulin resistance, FMT was performed using a microbiota from metabolically healthy lean donors or from the study participants themselves. A microbiota from lean donors significantly improved peripheral insulin sensitivity over that in controls. This change was associated with an increase in the relative abundance of the butyrate-producing bacteria related to *Roseburia intestinalis* (in the feces) and *Eubacterium hallii* (in the small intestine).

The efficacy of FMT for the treatment of recurrent *C. difficile* infection has been assessed in a number of small trials. One unblinded, placebo-controlled trial assessed the use of FMT in 42 patients with recurrent *C. difficile* infection (defined as at least one relapse after treatment with vancomycin or metronidazole for ≥10 d). Patients were pretreated with oral vancomycin. The experimental group then received FMT via nasoduodenal tube from healthy volunteer donors (<60 years of age) selected from the community. Controls underwent sterile lavage or received oral vancomycin alone. In 10 weeks of follow-up, infection was cured (with cure defined as three negative fecal tests for *C. difficile* toxin) in 81% of patients in the FMT group (13 of 16) but in only 23% (3 of 13) in the bowel-lavage control arm and 31% (4 of 13) in the vancomycin-only group. Metagenomic analysis of microbiota samples collected before and after treatment revealed an increased representation of Bacteroidetes and *Clostridium* clusters IV and XIVa, along with a 100-fold decrease in the relative abundance of Proteobacteria, in the FMT group.

A meta-analysis of FMT in *C. difficile* infection examined 20 case-series publications, 15 case reports, and the one unblinded study described above. All but one of these studies used fresh (not frozen) fecal samples. Donor selection varied, although most donors were family members or relatives and most studies excluded donors who had recently received antibiotics. It is noteworthy that the concentrations of infused donor feces varied widely (i.e., from 5 g to 200 g, resuspended in 10–500 mL); these fecal suspensions were introduced at different sites along the gastrointestinal tract, including the stomach and points throughout the small intestine and colon. Resolution of infection, which was frequently assessed on the basis of symptom resolution (with *C. difficile* toxin testing rarely performed), was documented in 87% (467) of 536 treated patients. The most common adverse events reported were diarrhea (94% of cases) and abdominal cramps (31%) on the day of infusion. The meta-analysis was limited to clinical outcomes and did not specifically address the role of the microbiota in disease resolution (e.g., the extent of invasion of donor taxa; their persistence; or the long-term effects of transplantation on various facets of host biology, which generally have not been evaluated).

Sober and thoughtful consideration needs to be applied to the therapeutic use of FMT, which represents an early and rudimentary approach to microbiota manipulation that very likely will be replaced by administration of defined collections of sequenced, cultured members of the human microbiota (*probiotic consortia*). A number of published reports on FMT have garnered significant public attention. This attention, coupled with an increasing public appreciation of the beneficial nature of our interactions with microbes, demands that the precautionary principle be honored and that risks versus benefits of such interventions be carefully evaluated.

To date, most FMT trials have failed to define (or have differed in) significant confounders, including (1) the criteria used for donor sample selection; (2) the methods used for donor sample preparation and characterization as well as the decision about whether or not to create a repository for donor and recipient samples that will permit retrospective analyses (and meta-analyses for given disease states); (3) the development of minimal standards for assessing the invasion of recipient gut communities by taxa from donor microbiota (using microbial source-tracking methods) as well as the timing, duration, nature, and breadth of sampling of the recipient as a function of transplantation; (4) the adoption of minimal standards for collection of patients' clinical data (e.g., age, diet, antibiotic use) and the establishment of databases for entering these data (including use of a defined vocabulary for annotating the clinical data); and (5) the development of standards for informed consent in lieu of knowledge of the long-term effects of the procedure. The regulatory landscape is evolving. The U. S. Food and Drug Administration recently issued an enforcement policy specifically addressing the use of FMT for the treatment of recurrent *C. difficile* infection; this policy indicates that the agency intends to "exercise enforcement discretion regarding the investigational new drug (IND) requirements for the use of FMT to treat *C. difficile* infection not responding to standard therapies," but it does not waive IND requirements for other FMT studies.

MOVING FORWARD

The design of human microbiome studies is rapidly evolving, in part because the data are highly multivariate, are compositional, and do not meet distributional assumptions of standard statistical tests such as analysis of variance. Consequently, the proper number of subjects to enroll and the proper populations to target remain to be established. One useful approach is to review published studies and ask whether the reported conclusion could be obtained with fewer subjects (*sample rarefaction*) and/or fewer sequencing reads from SSU rRNA genes, whole-community DNA (microbiomes), or expressed community mRNA (metatranscriptomes) per subject (*sequence rarefaction*). A common yet critical problem to avoid is under-sampling of the types of objects under study. For example, if the goal is to compare factors applying to individuals (e.g., individual diet), then dozens of individuals in each clinical category may be needed. If the goal is to compare factors applying to populations (e.g., demographic properties), then many populations may be needed.

Another key issue is whether the effect size to be studied, especially in meta-analysis, is greater than or less than technical effects. As noted above, different PCR primers will lead to different readouts of the taxonomy of a microbial community; these differences are, for example, greater than the differences between lean and obese subjects' fecal microbiota but less than the difference between fecal communities in newborns and adults.

A central challenge in human microbiome research is establishing the extent to which diagnostic tests and therapeutic approaches are generalizable. This challenge is illustrated by studies of the capacities of gut microbiomes to metabolize orally administered drugs. The results could be very informative for the pharmaceutical industry as it seeks new and more accurate ways to predict bioavailability and toxicity. However, these studies should prompt consideration of the fact that many clinical trials are outsourced to countries where trial participants have diets and microbial community structures that differ from those of the intended initial recipients of the (marketed) drug. Capture and preservation of the wide range of microbial diversity present in different human populations—and thus of the capacity of our microbial communities to catalyze elaborate and in many respects uncharacterized biotransformations—represent potentially fertile ground for the discovery of new drugs (and new industrial processes of societal value). The chemical entities that our microbial communities have evolved to synthesize in order to support their mutually beneficial relationships and the human genes that these chemotypes influence may become new classes of drugs and new targets for drug discovery, respectively. Therefore, characterization of groups of individuals living in countries that are undergoing rapid transformations in cultural traditions