Practical Aspects of Fecal Microflora Transplantation (FMT) for CDI

- Increased acceptance of FMT as a remedy to stop recurrent CDI
- Transition from individual donor / recipient pairs to universal donor model
- Donor screening and long term safety of donor derived microbes
- Multiple clinical trials examine methods to simplify delivery
- Transition to non-donor sources of microbes is needed
- Evolution of the strategy for FMT: stopping recurrent CDI now; examining a primary prevention model in the future.

Thomas Louie MD FRCPCH
dep. Med & Med Micro Immunol Infect Dis
Infection Prev. & Control, Alberta Health Services
Reuters) – Dec. 5, 2012. Drugmakers racing to develop medicines and vaccines to combat a germ that ravages the gut and kills thousands have a new challenger: the human stool.

For patients hit hardest by the bacterium Clostridium difficile, getting a "stool transplant" could become a standard treatment within just a few years. Just as blood banks and sperm banks are now commonplace, stool banks may soon dot the landscape.
Why FMT has taken off

- Fatigue in health care systems in dealing with recurrent CDI; antimicrobials not effective in stopping recurrences. Less tolerance of chronic illness by both public and physicians.
- Cultural acceptance & public awareness of “good” bugs, a foundation laid by probiotic usage and sales, and despite inconsistent data on efficacy.
- Increasing awareness of high efficacy of FMT for CDI
- Lifting of regulatory restrictions; more MD acceptance
- More awareness of pivotal role of microbiome in health
- Multiple methods of delivery improves patient access and acceptance.
Variables to consider in FMT

- **Selection and screening of donor and recipient**: the purpose is to protect the recipient, donor and provider from risks of transmission of pathogens and diseases.

- **Control of the symptoms of active CDI prior to FMT**. Most providers use vancomycin to attain disease control; fidaxomicin is theoretically better at preserving residual normal microbiota but the benefit over vancomycin is not established. It is recognized that vancomycin damages the microbiota but it is strategized that the incoming donor microbes will be sufficient to restore “colonization resistance”.

- **FMT for refractory patients**. From case series, FMT has been shown to be of benefit in patients who have persisting diarrhea disease despite antibiotic treatment. However, only a small percentage of patients should be uncontrolled. Maximized vancomycin oral dosages, IV metronidazole, and fidaxomicin or nitazoxanide should be used to achieve symptom control. A 7-14 day course is preferred to allow colonic mucosal healing in cases of active disease.
• **Bowel cleansing prior to FMT.** Most protocols use a standard colonoscopy bowel prep in the 24 hours prior to FMT to discard colon contents so that the incoming microbes will not be suppressed by residual concentrations of vancomycin or other antimicrobials; evacuation of the colon eliminates ecologic competitors. In the absence of a bowel prep, stopping vancomycin 3-4 days prior to FMT to reduce antibiotics in the gut lumen and infusion of a fecal slurry after a normal bowel motion works well.

• **Number of procedures.** Most protocols have strategized to use a single procedure to arrest recurrences, particularly when patients have endured multiple recurrences and months of illness and wish for the highest response rates to escape recurrence. However, no procedure guarantees success, therefore practitioners need to discuss the likelihood of needing a repeat procedure at the beginning of the consultation. The number of procedures needed is dependent on the dose response of the specific protocol.
Technical variables to be resolved: each FMT procedure has combinations of variables that are likely to affect outcome

- **donor microbial ‘quality’**—the composition of donor microflora, roles played in restoring colonization resistance, is unknown at present
- **quantity of microbes:** the concentration and volume of the inoculum to be delivered by each route. Dose ranging studies have not been performed in FMT trials to date. The most common volume in colonoscopy and enemas is ~400-500 ml of fecal slurry, range varying from as low as 50 ml to 1 Liter. Given that the response rate to arrest recurrences is ~85-90% with a single infusion in most cohorts, the benefit of high volumes to achieve a > 95% response versus risk of recurrence with lower volumes or quantity is still not established.
- **Route of administration:** Nasogastric/duodenal-jejunal, oral fecal capsules, colonoscopic delivery, enema
- **Criteria for outcomes evaluation:** efficiency at stopping CDI recurrences, adverse outcomes, cost, technical difficulty, patient convenience, provider convenience.
FMT pathway

Recurrent CDI $>3$

Treat CDI to achieve clinical cure

Bowel Prep with golytely, colyte, picosalax or wait 3-4 days for $>3$ bm’s

Donor microbes, find source, safety screening

Variable degrees of processing
- Blend whole stool in saline
- Filter wire mesh, gauze to remove particulates
- Add spin 300x g, pellet larger aggregates, discard, spin 10000x g for pellet→capsules
- Gelatin or acid resistant capsules
- Sequence pre-post transplant and donor feces to find consistently present post transplant microbes as candidates for restoration of colonization resistance. (Lawley)

Deliver the GOODS
Making FMT easier. Donors need to collect in a no touch fashion.
Donor selection

Exclusion of those with Infectious risk factors:

• Known HIV or viral hepatitis exposures
• High risk sexual behaviors
• Use of illicit drugs
• Tattoo or body piercing within previous 6 months
• Incarceration or history of incarceration
• Known history of tropical infection or current communicable diseases
• Other personal infectious disease risk factors including Creutzfeldt-Jakob disease (CJD)
• Travel history to endemic regions with a high risk of acquiring infectious pathogens
Donor exclusion

Exclusion of conditions that might affect health of the microbiome (examples).

- Gastrointestinal conditions (e.g., history of IBD, IBS, chronic constipation, chronic diarrhea, celiac disease)
- Atopy  e.g., asthma, atopic dermatitis
- Autoimmune conditions
- Chronic pain syndromes
- Metabolic syndrome
- Neurological conditions
- Psychiatric conditions
- Malignancy history
- Surgeries / Other medical history
- Medications including antibiotics, antifungals, antivirals, and immunosuppressants
- Family history (e.g., family history of IBD, colon cancer)
- Unusual or excess dietary habit
Donor Screening

Serologic testing:
- HIV antibody, type 1 and 2
- Hepatitis A IgM
- Hepatitis B (HBsAg, anti-HBc)
- Hepatitis C antibody
- Treponema pallidum
- HTLV 1 and 2
- Complete blood count with differential
- Hepatic function panel (AST, ALT, ALP, bilirubin, albumin)

Stool testing:
- PCR assay for C. difficile toxin B gene
- Culture for enteric pathogens e.g. *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, *Aeromonas*, *Plesiomonas*, *E.coli* 0157:H7
- *Helicobacter pylori* fecal antigen EIA
- Ova and parasites, full examination
- *Giardia lamblia* fecal antigen EIA
- *Cryptosporidium* fecal antigen EIA
- Acid-fast stain for *Cyclospora*
- Microscopy for *Microsporidia*
- Real-time PCR assay for fecal Norovirus, Rotavirus and Adenovirus
- Vancomycin-Resistant Enterooccus (VRE)
- ? Listeria monocytogenes
Additional QA practices to consider

- NGS of donor stool, Resistome
- Collection and Quarantine of donor stool to assure no illness in donor, verification beyond incubation period, with donor retesting
- Serial and continuous health monitoring of donors, illness management protocols
- Production/quality assurance practices for sample retention, quality control checks
- Monitoring of outcomes of FMT
- Recipient and donor registry to monitor health status over time
The beginning of commercialization, ~$250 plus ~$250 shipping; bulk buys

<table>
<thead>
<tr>
<th>size of poop</th>
<th># of people treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>50g</td>
<td>❌</td>
</tr>
<tr>
<td>100g</td>
<td>❌ ❌</td>
</tr>
<tr>
<td>150g</td>
<td>❌ ❌ ❌</td>
</tr>
<tr>
<td>200g</td>
<td>❌ ❌ ❌ ❌</td>
</tr>
<tr>
<td>250g</td>
<td>❌ ❌ ❌ ❌ ❌</td>
</tr>
<tr>
<td>300g</td>
<td>❌ ❌ ❌ ❌ ❌ ❌</td>
</tr>
<tr>
<td>350g</td>
<td>❌ ❌ ❌ ❌ ❌ ❌ ❌</td>
</tr>
<tr>
<td>400g</td>
<td>❌ ❌ ❌ ❌ ❌ ❌ ❌ ❌</td>
</tr>
<tr>
<td>450g</td>
<td>❌ ❌ ❌ ❌ ❌ ❌ ❌ ❌ ❌</td>
</tr>
</tbody>
</table>

THE MOST IMPORTANT THING
YOU'LL DO ALL DAY!
When to intercept with microflora restoration or augmentation

- **Primary CDI**
  - 20% risk of recurrence

- **1st recurrence**
  - 40% risk

- **1st recurrence despite taper of vancomycin**
  - ? No blinded RCT data

- **2nd recurrence with or without taper**
  - 60% risk

- **3rd recurrence**
  - 80% risk

- **≥ 4th recurrence**
  - Original intercept point
Enemas delivered at home, novel home help devices, balloon retention catheter is beneficial for those who have poorer anal sphincter tone.
How To Carry Out FMT
Transcolonoscopic infusion of 100-300cc liquid filtered flora
Reviews of FMT. Few RCTs, most small case series, variable definitions and practices.

<table>
<thead>
<tr>
<th>Study Details</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drekonja et al, Ann Intern Med, 162: 630-8, 5 May 2015</td>
<td>2 RCTs, case series, 655 patients reviewed</td>
</tr>
<tr>
<td>National Institute Health &amp; Care excellence (NICE, Scotland) 2014 Mar.</td>
<td>289 patients in 25 series.</td>
</tr>
<tr>
<td>Am J Gastroenterol 2013 Kassam et al</td>
<td>273 patients</td>
</tr>
</tbody>
</table>

Inoculum volume reported highly variable from 25 gm or ml to 960 ml, average ~350 ml.

**Concensus:** it appears that recurrence is prevented ~85-90% of the time with colonoscopic delivery, that an oral route is slightly less effective in case series to date. Variations in practice and non-reporting of variables that are expected to affect outcomes need to be further researched.
BL, 83yo Female: **Oral** Transplant Gut Microflora Profile: 10 caps QD x4, Jul 2010. Ten capsules/d [=4.7ml] for 4 days. No recurrence but C.diff regenerates. This observation prompted the strategy to deliver microbes in one session.
Calculation of microbial inoculum for oral capsule FMT

- Assume log 11.5/ml fecal microbes = 100 Billion/ml
- 0.47 ml/capsule #1 size
- x 30-40 capsules = 14-19 ml / treatment
- However, acid exposure likely reduces counts
- After 90 minutes acid exposure pH 2.5 as a single spot measure of the effect of HCl on microbial survival, neutralized sample, reculture: On 10 normal fecal samples, Enterococcus spp CFU log 3.58 → < 2.0
  \[
  E. \ coli \quad 6.31 \rightarrow 4.39
  \]
  Bacteroides spp. \quad 9.80 \rightarrow 8.28
- mean 1.5 to 2.0 log reduction with acid exposure, possibly more depending on gastric emptying
- calculated dose= 1-2 Trillion less acid reduction = approximately 50-100 billion microbes; it is possible the spore component of the inoculum contributes to the FMT benefit as $10^{7-8}$ CFU/gm are recovered after alcohol shocked fecal microbes.

Double over encapsulated capsules with fecal microbes plus methylene blue did not release dye until ~ 2 h at RT, ~ 1 h at 37° C. X ray of BaSO₄ marked capsules at 90 minutes post ingestion implies dissolution of capsules, exposure to acid.
FMT by oral capsules to date:

• 55 patients, July 2010 to present. 3 or more episodes of rCDI.
• 5 initial patient received antacid medication, but the subsequent patients have not received antacid medication for the transplant.
• 2/55 recurred, at 1 and 2 months, both received retreatment.
• Patients with underlying UC appear to respond also
• Overall well tolerated, gastric fullness in most; nausea in several patients, no persons vomited, patient are instructed to remain NPO for 2.5 hours post ingestion, diarrhea for ~1 day in <10% patients. Serial stool follow up at 1 week, 1 month, 3 months, 1 year
• 1-4 year follow up pending.
qPCR analysis of the gut microflora in *C. difficile* infected patients pre and over time post bacteriotherapy, n=27. Pre therapy microflora levels are compared to (A) 1 week, (B) 1 month, (C) 3 months and (D) up to 1 year post bacteriotherapy. CFU, colony forming units,* p<0.05. [Louie, unpublished]
Clinicaltrials.gov....37 trials registered as of May 2015. Examples:

<table>
<thead>
<tr>
<th>NCT01868373</th>
<th>Variation of Michel Tvede’s 10 microbe cocktail by ugi tube at Baylor, Houston [ S. fecalis, C. innocuum, C.ramosum, C. bifermentans, P. productus, B. ovatus, B. vulgatus, B. thetaiotamicron]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02343328</td>
<td>FMT after First Recurrence: capsules vs placebo capsules; Mass. General, Dr. Hohmann</td>
</tr>
<tr>
<td>NCT01703494</td>
<td>Donor stool versus patient’s own stool, re-infused as sham Dr. Colleen Kelly, Rhode Island, n=53</td>
</tr>
<tr>
<td>NCT02254811</td>
<td>Fecal capsules vs colonoscopy, from 100 gm stool, Alberta n=100</td>
</tr>
<tr>
<td>NCT02299570</td>
<td>Punch 2, RBX2260 vs placebo by enema, DB, retreatment if recurrence. N=117</td>
</tr>
<tr>
<td>NCT02133651</td>
<td>Emory, 20 patient cohort for stool and mucosal metagenomics</td>
</tr>
</tbody>
</table>
Shifting to component microbes for FMT.

- Trevor Lawley shows that 6 microbe combination eradicates carrage in mice [Plos Pathogens, October 2012]
- Reeves –Young shows lachnospiraceae protect against C. difficile in mice [ Infect Immun, Nov. 2012]
- Re-poopulate works in 2 patients [ Microbiome 2013]

$10^9$ spores from fecal source, >90% prevention of recurrence, clearance of nosocomial gut pathogens. This phase 2 study shows potential of using components of the microbiota.
Repopulate, composition of stool substitute, 33 microbes.
Emma Allen-Vercoe and Elaine Petrof, U Guelph and Queens U, respectively. Microbiome 2013.

- Acidaminococcus intestinalis
- Bacteroides ovatus
- Bifidobacteria adolescentis (2)
- B. longum (2)
- Blautia producta
- Clostridium cocleatum
- Collinsella aerofaciens
- Dorea longicatena (2)
- E. coli
- Eubacterium desmolens
- E. eligens
- E. limosum
- E. rectale (4)
- E. ventriosum
- Faecalibacterium prausnitzii
- Lachnospira pectinoshiza
- Lactobacillus casei
- Lactobacillus casei/paracasei
- Parabacteroides distasonis
- Ruminococcus torques (2)
- Ruminococcus obeum (2)
- Raoultella sp.
- Roseburia intestinalis
- Roseburia fecalis
- S. mitis

Combination of these microbes are first grown in Dr. Emma Allen-Vercoe’s lab in Guelph, Ontario, then driven* to Kingston General Hospital for deposition in colon. Cures 2 patients. NCT 01372943, * 340 km
Dr Emma Allan-Vercoe, Guelph and the human ‘robo’ gut model,

From Canadian Broadcast Corporation, 2011
Freeze-dried, Capsulized Fecal Microbiota Transplantation for Relapsing Clostridium difficile Infection

Hongliang Tian, PhD*
Chao Ding, MD*
Jianfeng Gong, MD*
Yao Wei, PhD*
Lynne V. McFarland, PhD†
Ning Li, MD*

*Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, China
†Department of Medicinal Chemistry, University of Washington, Seattle, WA

Comment: first case of an RCT, representing a lot of pressure to publish findings
When to intercept with microflora restoration or augmentation?
Currently at 3rd and 4th recurrences, but there should be a movement to consider interception at 2nd recurrences. If component microbes are available by capsules, it would be possible to restore or recomplement the microflora in patients receiving microbiome damaging antibiotics eg vancomycin at primary and first recurrences.
When to intercept with microflora restoration in the general hospital? It is become increasingly attractive to consider modified FMT procedures in a preventative fashion since a large proportion of the infection control challenges in hospitals can be traced back to a depleted microbiome allowing nosocomial pathogens to flourish.

Primary CDI

1st recurrence

1st recurrence despite taper of vancomycin

2nd recurrence with or without taper

3rd recurrence

≥ 4th recurrence

Measuring the impact of antimicrobial agents on the microbiome and restoration to a healthy state by replacement of damaged components should prevent CDI from occurring. This is the new frontier of microbiome therapy. VRE, fungal overgrowth, Cdiff and proteobacteria overgrowth should theoretically be limited.
Conclusion: Where are we with FMT?

- We are in a transitional state, shifting away from higher cost endoscopic delivering of raw feces over the next few years. Enemas and NG/jejunal tubes continue to be used as well.
- Transition to oral approach. If there is a small bowel component of \textit{C. difficile} involvement, an oral or upper GI route may be beneficial.
- It appears that whole fecal composition not necessary to prevent \textit{C. difficile} recurrences.
- Component microbe reconstitution and serial measurement of microbiota reappearance would elucidate differential role of microbes. Currently it appears that FMT does repopulate with donor organisms, but also encourages low numbers of persisting recipient flora to regenerate.
Conclusion 2: FMT

- Fecal capsules should make it possible to prevent recurrences in patients receiving systemic antimicrobial treatments, rather than to use metronidazole or vancomycin prophylactically.
- FMT is on the threshold of becoming a common practice in healthcare to bolster, restore microbiome in a variety of health conditions as a preventative approach. Microbiome health status should become a hospital test.
- Final comment: Narrow spectrum *C. difficile* therapies, here now, and more coming, should reduce recurrence burden.