

Update on *Clostridium difficile* Research: Hospital Reservoir, Diagnosis of CDI and Harnessing Intestinal Microbiota

TDSHS Sponsored Research

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President, Kelsey Research Foundation

Additional appointments as Professor:

University of Texas and Baylor Graduate Schools of Biomedical Science

MD Anderson Cancer Center

University of Houston

Conflicts

- **Grants for clinical studies:**

**Sanofi Pasteur,
Takeda, Seres
Health, Riobitex
Salix-Valeant**



Disinfection Methods

- Ultraviolet light plus standard treatment (quaternary ammonium solution)
- 10% bleach (Dispatch)
- Hydrogen peroxide/Peracetic acid (Oxycide)
- Quaternary ammonium solution only as a control group

Study Floor	Method of Decontamination
Floor 1	Ultraviolet light plus quaternary ammonium solution (odd numbers)
	Quaternary ammonium solution ONLY (control) (even numbers)
Floor 2	10% Bleach (Dispatch)
Floor 3	Hydrogen peroxide plus peracetic acid (Oxycide)

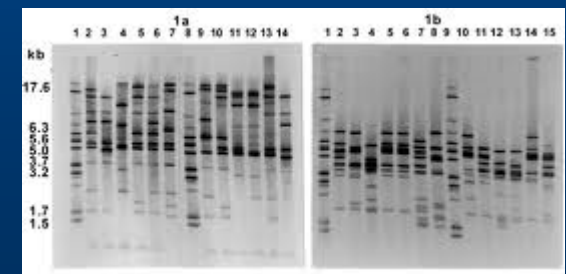
C. difficile Identification

3 Hospital Floors Studied

- Environmental sponge swabbing patient room and bathrooms and stools samples from patients with diarrhea were cultured for *C. difficile* using and confirmed by PCR
- Molecular typing method used was to look for clustering suggesting hospital-acquired infection vs single clones suggestive of community acquisition



Cycloserine-cefoxitin fructose agar



multilocus sequence typing (MLST)

Study 1 Design

- **3 hospital floors with a total of 85 rooms housing 126 adult patients were sampled**
- **Swabs were from high touch areas of patient room and in bathroom around toilet while rooms were cleaned daily**

C. difficile Identified from Rooms of 3 Study Units Using Different Cleaning Methods July 2014 through June 2015

Clean Method	N	Room with <i>C. difficile</i> Identified	Bathroom with <i>C. difficile</i> Identified	Total Rooms with <i>C. difficile</i> Identified
H ₂ O ₂ & peracetic acid (OxiCide)	802	17 (4%)	51 (13%)	60 (7%)
Quaternary Ammonium	468	5 (2%)	20 (9%)	23 (5%)
UV plus QA	548	3 (1%)	11 (4%)	12 (2%)
10% Bleach	996	13 (3%)	35 (7%)	44 (4%)
Total	2814	38 (3%)	117 (8%)	139 (5%)

QA = Quaternary Ammonium, UV = UltraViolet

P value= 0.0003, when comparing *C. difficile* contamination rate in the hospital environmental with different cleaning methods

Study 2 Design

- All strains of *C. difficile* isolated from surface of study rooms were typed by MLST* to identify clones
- Molecular fingerprinting can identify in-hospital transmission of strains

*Multi-locus sequence typing

Clone

70% Similarity

1

Floor (Positive/Total) P Value

3 (6/67=9%)

1 (6/39=15%)

2 (15/47=32%) ✓

0.0061

2

3 (53/67=79%) ✓

1 (12/39=31%)

2 (26/47=55%)

0.0001

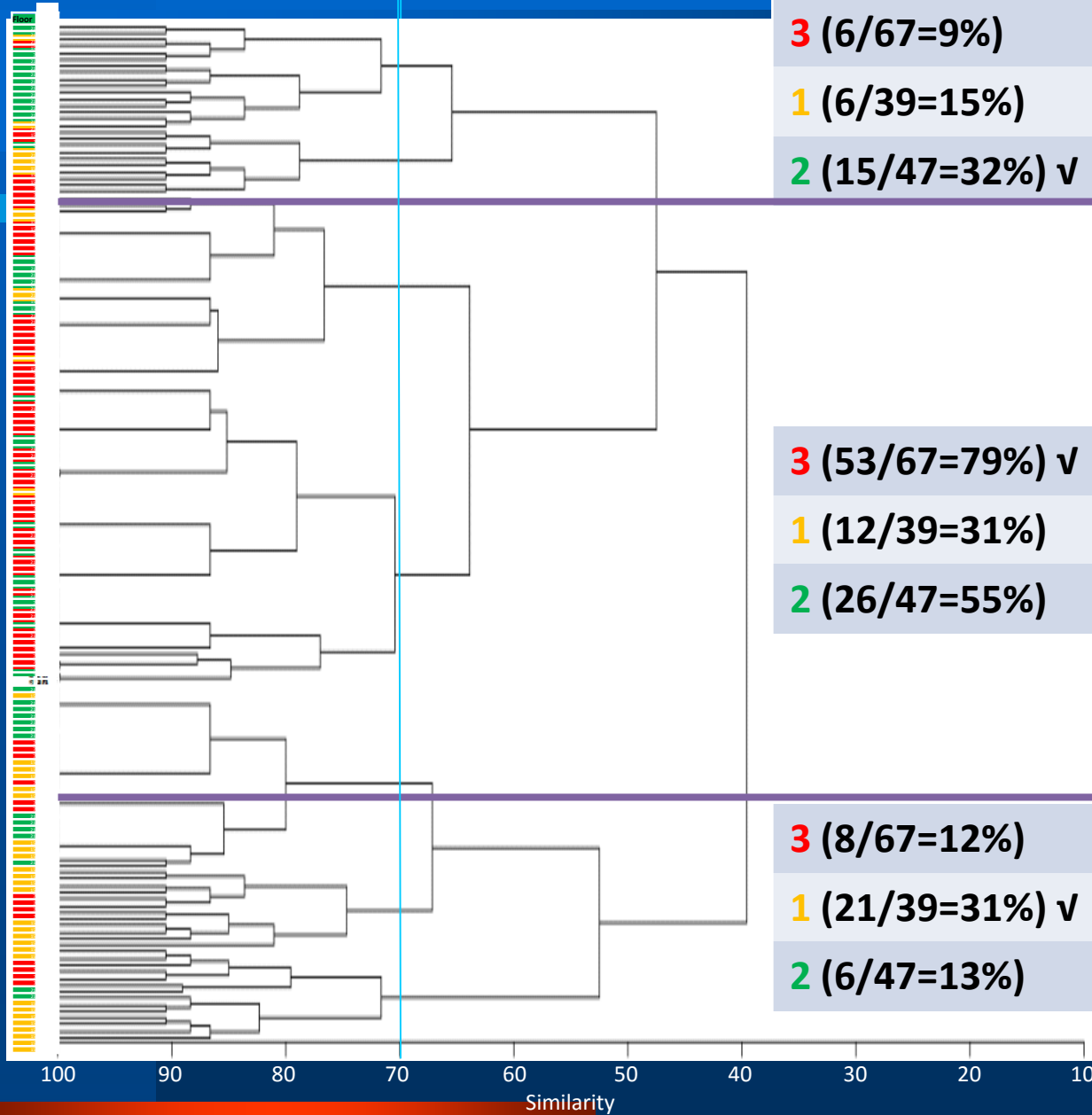
3

3 (8/67=12%)

1 (21/39=31%) ✓

2 (6/47=13%)

0.0001



Conclusions Study 1 and 2



- Ideal cleaning is quaternary ammonia + UV light plus which poses challenges because of time with room not in use
- It may be that any cleaning method will work if hospital housekeepers spend sufficient time cleaning
- Much of hospital-associated CDI is acquired regionally in hospital units where clones of spores can be found
- Multiple clones are seen throughout the hospital from multiple entry points

Clostridium difficile – a Unique Hospital Problem



- Hands of 24% of health care workers positive for spores vs. 0% for hospital workers without patient exposure¹
- *C. difficile* spores are shed from both symptomatic/asymptomatic patients
- 4%-13% inpatients without diarrhea are colonized by *C. difficile*^{2,3}
- Study found that contact isolation of carriers admitted from the ED led to significant reduction in CDI in the hospital²
- Spores can survive months without killing by standard disinfectants

¹Landelle C et al. Infect Control Hosp Epi 2014;35:10-15

²Longtin Y et al. JAMA Intern Med 2016 Epub

³Koo H et al. Infect Control Hosp Epidemiol 2014;35(6):35:667-73

Modern Methods of Infection Control



- We have to focus on both endogenous and exogenous pathogens
- Room cleaning needs to be performed by dedicated and trained personnel
- UV radiation, gaseous plasma (hydroxyl and hydroperoxyl free radicals) or hydrogen peroxide vapor may help
- Antibiotic stewardship offers major payoff
- The Hospital Microbiome Project¹ takes an ecological approach to improve intestinal colonization resistance to reduce environmental transmission of pathogens and antibiotic resistance

¹Shogan BD et al. *Stds Genomic Sciences*
2013;8:571-9

How to Modernize Hospital Infection Control

- Require an accurate diagnosis of CDI and stop treatment if found negative
- Assume all patients and hospital personnel are *C. difficile*-positive
- Require that room cleaning be effective and that frequent and effective hand washing with soap and water are performed
- Stay tuned to ongoing studies of disinfection of hospital environments



Study 2 Difficulty in Diagnosing CDI

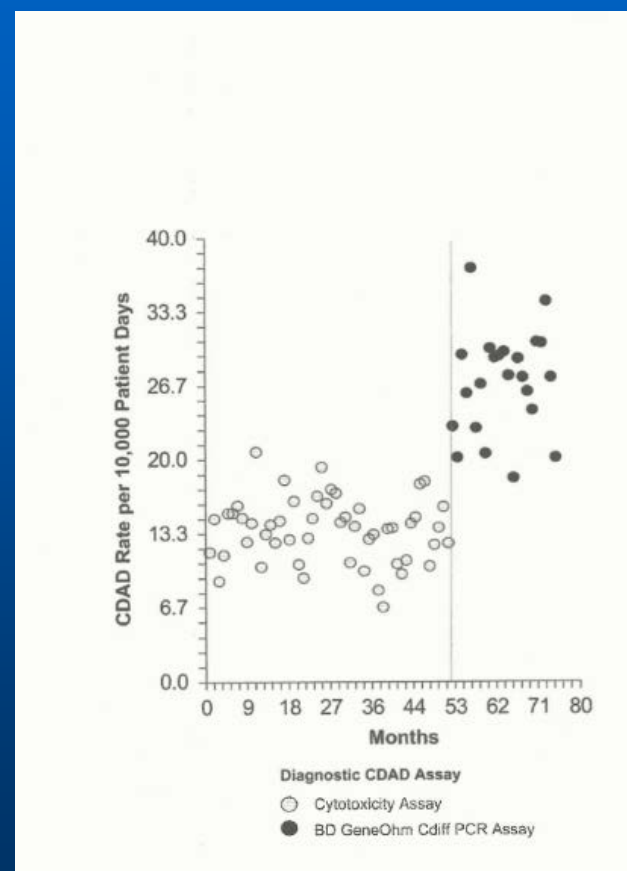
Making the diagnosis: diarrhea plus positive fecal test for *C. difficile* toxin(s):

- EIA lacks sensitivity;
- Toxigenic culture and Tissue culture cytotoxicity assay takes 3 days
- PCR overly sensitive and picks up carriage
- Two step methods have been developed (e.g. glutamate dehydrogenase + EIA or PCR)
- Fecal CD toxin test plus finding inflammatory markers in stool suggests; Finding pseudomembranous colitis by endoscopy confirms the diagnosis



PCR Identification of CDI

- When in May 2011 our 700-bed university hospital in Houston changed from cytotoxicity assay to PCR the rate of CDI doubled from 13.4 to 27.0 patient days/10,000.
- PCR+ *C. difficile* toxin(s) found in 13% of asymptomatic patients in the hospital
- 10% of patients in our hospital with AAD are *C. diff* toxin positive
- Most antibiotic associated diarrhea in our hospital with positive PCR test for *C. difficile* toxin is a false positive



Koo H et al. *Infect Control Hosp Epidemiol* 2014;35(6):35:667-73

Making an Accurate Diagnosis of CDI

- Most patients with antibiotic-associated diarrhea don't have CDI
- Colonization of C diff is seen in between 5-15% of inpatients
- Considering these most PCR positive C diff's are false positives
- We are looking at 170 patient stools positive for *C. difficile* for presence of inflammation markers (calprotectin and lactoferrin)



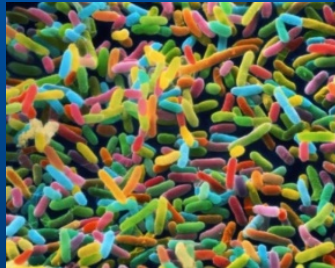
Outcomes Studied

- With a gold standard diagnosis of CDI we can educate doctors to treat those and avoid false positives
- Laboratories may perform PCR plus neutrophil inflammation marker reporting ++, +-, --



Healthy Diverse Gut Microbiota

Healthy Eating and Avoiding Unnecessary Antibiotics



The packed microbes (10 times the number cells in the body) prevent growth of antibiotic resistant bacteria and disease causing bacteria (e.g. *Salmonella* & *C diff*) because of space the healthy bacteria occupy & organic acids they produce

Unhealthy Diet and or Recurrent or Prolonged Antibiotic Exposure

Sparse Intestinal Microbiota = Dysbiosis



Blooms of unhealthy
Proteobacteria



Few bacterial species
giving room for harmful
bacteria to grow

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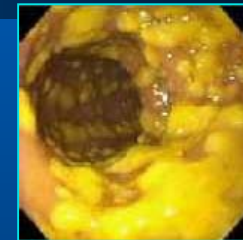
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Proteobacteria



Few bacterial species
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With exposure to antibiotics
and exposure to hospital
spores, *C. difficile* can grow
unchecked leading to severe
potentially fatal diarrhea

Clostridium difficile
infection



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With exposure to antibiotics
with dysbiosis *E. coli* can
grow unchecked lead
antimicrobial resistant
intestinal microbiota leading
to difficult to treat infections



Antibiotic
resistance

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Sparse Intestinal Microbiota = Dysbiosis



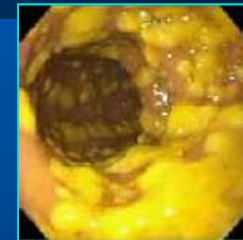
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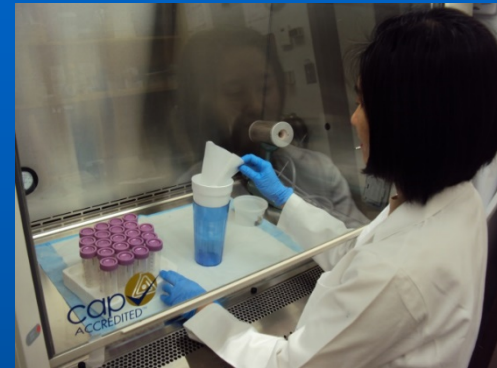
Antibiotic
resistance

Fecal Microbiota Transplantation (FMT) can reverse dysbiosis

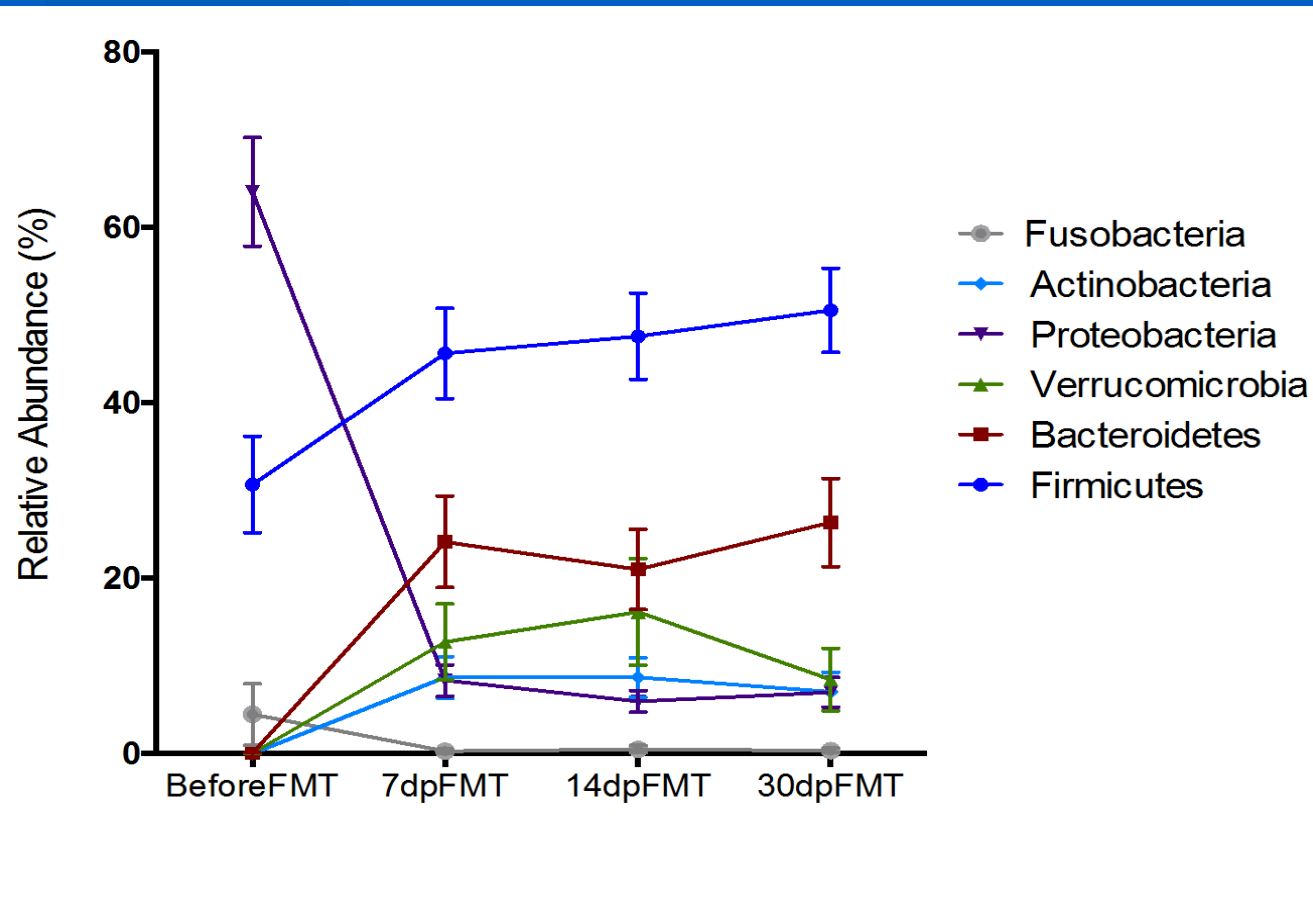
For Depletion of Diversity of Microflora - Fecal Microbiota Transplantation (FMT)

Donor Screening and Preparation & Delivery of FMT Product

- Donor selection and screening process
- ≥ 100 g of stool is collected and mixed in stomacher and filtered twice to remove fecal material
- Study 1: fresh, frozen or lyophilized (freeze-dried) bacterial product dispensed via colonoscope
- Study 2: frozen product given by enema and lyophilized by oral capsule
- 90% successful with one treatment

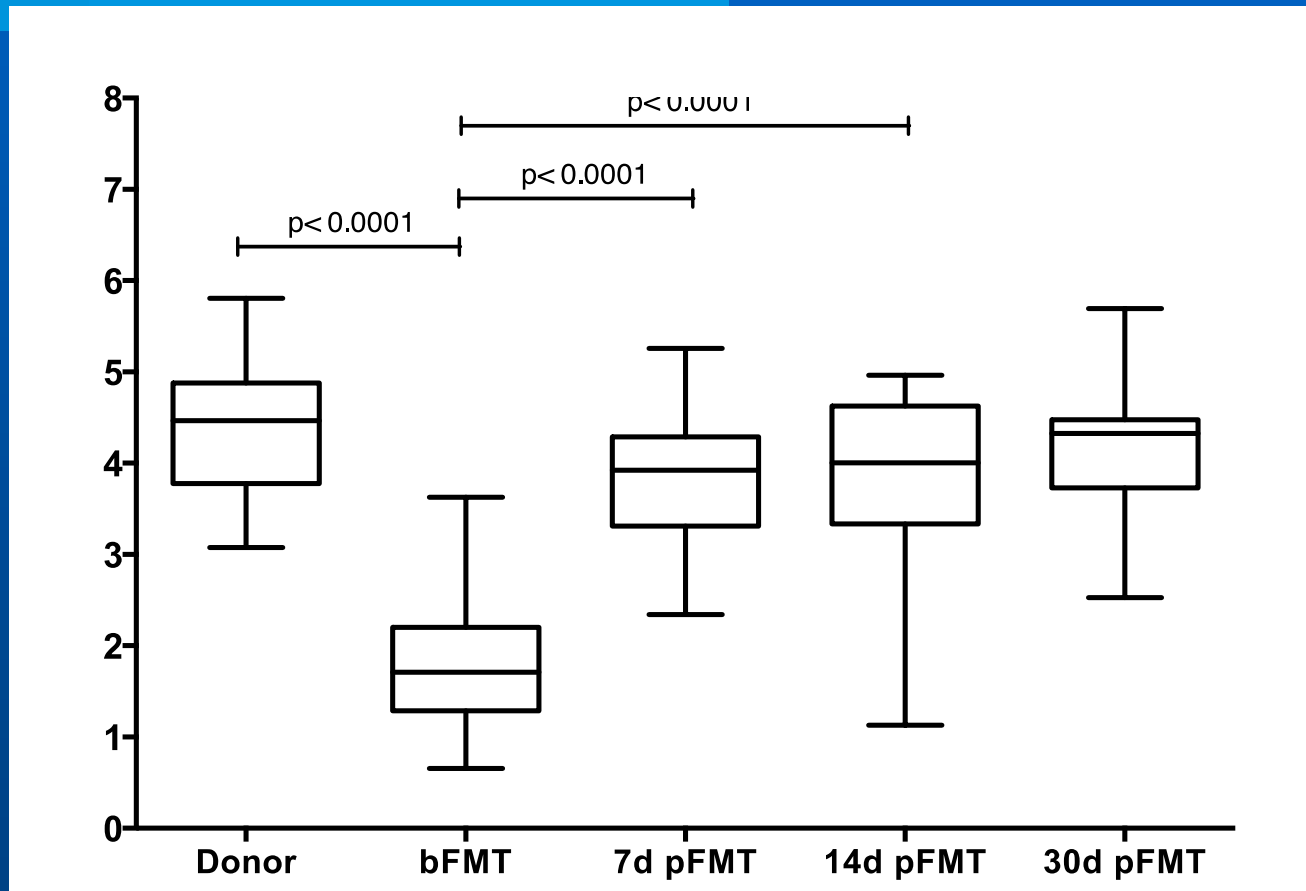


Fecal Microbiota in Recipients Before and after Fecal Microbiota Transplantation



Recipients After ≥ 3 Bouts of CDI

Fecal Flora Diversity in 8 Donors and 41 Recipients Before and After Fecal Microbiota Transplantation



Recipients After ≥ 3 Bouts of CDI

Our Patients Remind of Us of the Miracle of FMT

- We have treated more than >120 patients with recurrent C diff infection by FMT

- Success rate 90%; failures receive second FMT from a different donor

- Our patients will show you how grateful they are



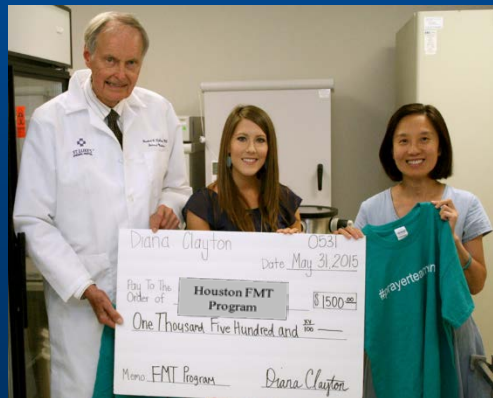
FMT # 1-9

“Thank you!, Thank you!, Thank you!
For the 1st time in a year I can hold
a grandchild”



FMT # 1-69

Chairman, Department Head, sent the university his resignation, cured by FMT, rescinded his resignation, “you saved my life”



FMT
2 #10

20-yr old lost 40 lbs (down to 88 lbs), was cured by FMT and organized fund raising event to pay for medical expenses



FMT
1 #69

19-yr old college football player with ulcerative colitis and 4 episodes of C diff infection with 90 lbs of weight loss was cured by FMT and he is back playing football

Partial List of Conditions With Unhealthy Dysbiosis of Intestinal Flora

- *Clostridium difficile* Infection
- Inflammatory bowel disease & Irritable bowel syndrome
- Type II diabetes and metabolic syndrome
- Rheumatoid arthritis
- Chronic neurologic diseases (e.g. MS, Parkinson's disease)
- Allergic disorders and asthma
- Allogeneic hematopoietic stem cell transplantation
- Obesity
- Hospitalized patients & resistant microbiota



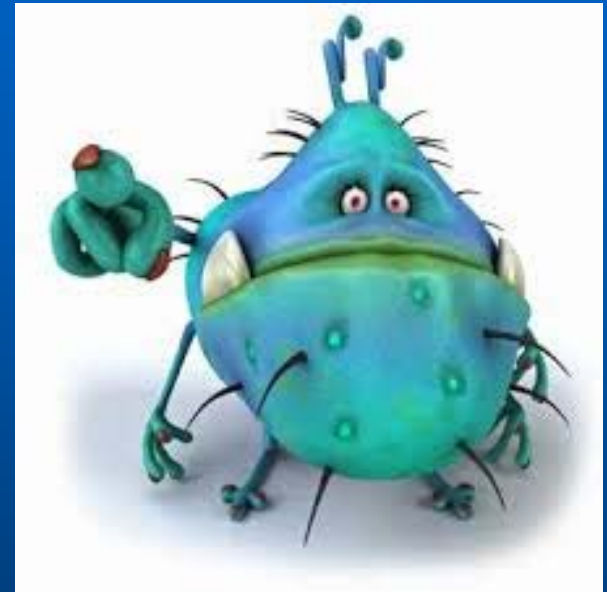
Control of Current Epidemic of Antibiotic Resistance

- Key factors: avoid unnecessary antibiotics (Stewardship)
- Use narrow spectrum antibiotics when can
- Maintain a healthy gut microbiota with diet
- Fecal microbiota transplantation may reverse MDR colonization



Conclusions and Summary

- Careful cleaning of hospital rooms is critical to controlling C diff infections: UV light plus conventional cleaning is effective but more rigorous standard cleaning may work
- Being aware of the problem of false positive testing for CDI should lead to better antibiotic stewardship by focusing on those with clinical disease
- A gold standard diagnostic test is needed
- FMT is a powerful low-tech physiologic means of reversing dysbiosis that is being harnessed to treat non GI diseases & potentially prevent endogenously acquired infections from gut microbiota and reduce the reservoir of antibiotic resistance



Support Bacteria
They are the Only Culture
Some Folks Have!