Fecal Microbial Transplantation as an Alternative Treatment for Recurrent *Clostridium difficile* Infection

Anam Qudrat, Institute of Biomaterials and Biomedical Engineering (IBBME), University of Toronto

Abstract

Clostridium difficile infection (CDI) is a bacterial infection, most often contracted in the hospital. With symptoms ranging from mild diarrhoea to severe gut inflammation, it has become an emerging epidemic in the last decade. As the infection rate increases alarmingly, it becomes a burden on patients and their healthcare providers to manage. Coupled with this increase is the imminent threat of recurring episodes, termed recurrent *Clostridium difficile* infection (RCDI). As hypervirulent strains evade conventional drug therapies, investment in fecal microbial transplantation (FMT) becomes a viable alternative. Specific advantages of the approach over conventional treatment are its relative efficacy and cost effectiveness. Here, we survey this procedure, its current implementation, and future prospects.

Clostridium Difficile Infection at a Glance

Clostridium difficile infection (CDI) is a nosocomial (or one that is contracted in the hospital) pathogen, causing diarrhoea. It has become a more rampaging epidemic than even Methicillin-resistant *Staphylococcus aureus* (MRSA), causing as many as five times the number of deaths in the past two decades. Of diseases caused due to enteric pathogens, *C. difficile* accounts for 73%.[1–4] Along with being contracted in the hospital, over the past decade, community-based acquisition has increased.[5, 6] Symptoms range from mild diarrhoea to a severe pseudomembranous colitis where the colon is greatly inflamed.[7,8] Reported cases are on the rise in the developed as well as the developing world, spreading from person to person through physical contact, mainly in the elderly population.[9–11] These incidences cannot be simply explained by citing improved CDI detection and analysis assays, thereby affirming that there is a legitimate threat presented by this infection.[12] A longitudinal study in the United States that measured hospital short-stays for *C. difficile* associated diseases (CDAD) from 1996 to 2003 documented the discharge rate nearly doubling over the seven year period, with significant increases in the years 2001 and 2003.[1,13] Although there was no significant difference between males and females, the rate did increase in elderly patients (> 65 years), especially in intermediate- or large-sized hospitals (>100 beds).[1] A 23% annual increase in hospitalizations due to CDAD was reported between the years 2000 and 2005.[13] Whereas in the early 2000s, the discharge rate due to CDI was reported as 3.82 per 1000 discharges, this number increased to 8.53 per 1000 discharges in 2009 in North America.[14] Furthermore, the Canadian Nosocomial Infection Surveillance Program cites an increased rate of 4.51 to 5.35 CDI per 1000 patient admissions from 2007 to 2011 respectively.[15] Thus efforts to combat and limit CDI spread are well substantiated.

This rising infection rate, in addition to depreciating the patients' quality of life, weighs heavily on the health care system. A cohort study of 271 patients found that, of the 15% that contracted CDI, their hospital stay became twice as long (with 3-20 extra hospital days added) and the cost per patient increased by 54%.[16,17] Based on this, Kyne et al. attributed estimates of >$1.1 billion of excess costs to CDAD.[16] A recent study in 2012 reviewed mortality rates due to CDI in North America and Europe. Based on 19 publications, it concluded that mortality after 30 days ranged from 9-38% in Canada alone reporting an average mortality rate of 22%.[18-20] Despite the heterogeneous nature of study design and data collection amidst these publications, the disturbing rate of mortality clearly highlights the severe consequences of this disease.

Where Recurrent CDI Makes its Mark

A recurring episode of CDI is termed recurring episodes of *Clostridium difficile* infection (RCDI) – a major complication for both patients and health care practitioners. The reoccurrence can be characterized as *relapse* where the same strain infects the patient again or *reinfection* where another strain infects the patient.[21] Whereas the reoccurrence after one episode of CDI can range from 15-45% of the patients, it increases to 65% after subsequent cases. These rates are on the rise since 1988.[3,17,20,22] The recurrence rates were similar irrespective of the drug therapy used for treatment, most prevalently metronidazole or oral vancomycin.[7,9] Although mean ages did not significantly differ, a study reporting 45% relapse rate showed that females were more likely to re-

Corresponding Author:
Anam Qudrat
Rosebrugh Building, Rm 404
164 College Street
Toronto, ON M5S 3G9
anam.qudrat@mail.utoronto.ca

lapse.[22] Also reported was that the duration of recurring episodes is significantly longer and usually occurs within 2-10 days of the initial treatment but can do so within two months as well.[3,21,22] Increasing risk of mortality and longer hospital stay times furthers the disease's health as well as economic burden.[3,10,16]

Disease Pathophysiology

C. difficile is an anaerobic bacterium that forms endospores conferring resistance to harsh environmental conditions. The virulent spread of the species within the host creates an imbalance between the beneficial and harmful bacterial species in the gastrointestinal tract, referred to as dysbiosis.[23] In addition to causing recurrent *C. difficile* infection, there is a 5 – 32% chance that it can lead to diseases such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).[24,25] In the case of *C. difficile*, upon colonizing the gut, it will release toxins causing diarrhoea and trigger an inflammatory response.[26] Disease severity ranges from diarrhoea and abdominal pain to fulminant colitis, or a severe form of inflammation in the inner lining of the colon.[8]

Genetically encoding and regulating the toxins is a 19.6 kilobase pair locus. This codes for toxin A (*tcdA* gene), toxin B (*tcdB* gene), their transcriptional up-regulators (*tcdR* gene) and transcriptional down-regulators (*tcdC* gene). Both toxins show considerable sequence homology with an N-terminal glucosyltransferase domain, a central hydrophobic translocation domain, and a C-terminal receptor binding domain.[9] After cell surface receptor binding, the toxin protein undergoes receptor-mediated endocytosis. Enclosed within the endosome, the acidic environment induces a conformational change resulting in pore formation of the central hydrophobic domain. This causes release of the N-terminal glucosyltransferase domain into the cytosol which following a second conformational change undergoes auto-cleavage and releases the glucosyltransferase segment. In effect, this catalytic component triggers a signal cascade through Rho GTPases disrupting the cytoskeleton which subsequently leads to cell death.[27]

Coupled with persistent survival in the gut is the emergence of *hypervirulent strains*. To note is the ribotype 027 strain and a newly discovered 078 type, which are resistant to quinolone antibiotics and have led to a five-fold increase in CDAD prevalence over the past decade.[20,28] Whereas other strains are known to produce two types of toxins, A and B, this particular strain additionally produces accessory binary toxins and other virulent factors that allow it to adhere to and colonize the intestinal mucosa more efficiently.[9,28-30] This is thought to act synergistically with toxins A and B during the pathogenesis.[9]

With an intact colonic flora or microbiome population, healthy adults are resistant to the disease. However, with a perturbation in either the composition or the survival of various bacterial strains, susceptibility to the disease increases. Patients are often more susceptible to RCDI due to old age, weak or immunosuppressed systems, low serum albumin level, surgery, and repeated antibiotic use.[4,8,21] Of these, the use of broad spectrum antibiotics serves as the most potent risk factor as it markedly reduces the *colonization resistance* or the innate ability of the functional microbiome to fight off foreign agents.[4]

Drugs as the Conventional Treatment Option

Currently, there are three main treatment options for CDI: antibiotics against *C. difficile*, probiotics to reduce growth of infectious bacterial strains, and intravenous immunoglobulins to build antibodies again toxins A and B.[9] The two drugs primarily used for antimicrobial therapy are metronidazole or oral vancomycin.[9] One randomized, double-blind, placebo-controlled study comparing the efficacy of both of these drugs showed that although both were effective in treating mild CDAD, vancomycin performed better in treating severe disease.[7] In those suffering from severe CDAD, 97% as compared to only 76% had symptoms alleviated, when treated with vancomycin and metronidazole respectively, with a 15% relapse rate.[7] Based on the number of recurrences and the severity of the disease, treatment regimens have been prescribed in clinical guidelines.[4,9,26] Instead of direct dose administration, pulsed or tapered regimens of vancomycin have been suggested resulting in significant decreases in RCDI.[22] After the antimicrobial treatment is complete, patients are at a risk of relapse as the drugs upset the microbiome making it more susceptible to infection by the prevailing *C. difficile* spores.[26] Also considering bacterial resistance, the efficacy of metronidazole is depreciating over time as less susceptible strains are evolving.[21,28,31] However, vancomycin is often used as a last resort since it is a broad antimicrobial agent acting against all gram positive aerobic and anaerobic bacteria – specifically it is a glycopeptide antibiotic that destroys gram positive bacteria cell walls.[32] It is clear from this that these drugs sustain an altered microbial flora in the gut, which in turn heightens susceptibility to future infections. Changes in drug distribution, variability in symptom analysis and disease classification, underpowered studies, side effects such as pain (in the bladder, muscle, and/or back), difficulty urinating, nausea or vomiting amidst others, and the use of observational studies with their inherent biases should call to question drug use as a treatment option overall.[6,20]

Using probiotics is another therapeutic option to restore the microbial flora of the gut. Administering probiotics in combination with antibiotics, most commonly of the lactobacillus species like *Saccharomyces boulardii* show great promise in treating antibiotics associated diarrhoea (AAD) but do not show consistent results for CDI.[9,33–35] Drawbacks include using high dosage drug therapies alongside these treatments and the danger of developing fungemia.[21] Lastly, although intravenous immunoglobulins have shown promise in increasing the serum antibodies against toxins A and B, this approach has not been evaluated in a large, controlled clinical trial and thus remains elusive.[9,36] With this type of immune therapy, the microbial infectious agents are not eradicated so there is still a potential threat residing in the body – a major reason hindering its wide acceptance. Coupled with high costs and limited evidence, immune therapy is not a feasible treatment option at the moment.

As new drugs such as Tolevamer (a *C. difficile* toxin-binding agent) are launched into the market, their efficacy in treating primary CDI is put into question. Although Tolevamer treatment reduced recurrence rates slightly by 20% (compared to vancomycin) and 24% (compared to metronidazole), it had a significantly lower cure rate for primary CDI.[9,37] Moreover, with a large proportion of the genome carrying antibiotic resistance genes, *C. difficile* strains are likely to evolve and resist drug treatment. When, alas, the costly conventional treatments prove ineffective, moving towards fecal microbial transplantation (FMT) as an alternative treatment route becomes an option.[38] Although not a widely accepted approach, it does aim to restore the intestinal microbiome community facilitating self-repair.

Opening Doors to an Alternative Approach: Fecal Microbial Transplantation (FMT)

For severe, refractory infection, the alternative treatment is a form of bacteriotherapy. The rationale for fecal microbial therapy is to re-establish a healthy gut microbiome which effectively dissuades the rampaging infection – a diverse community creates a system of checks and balances to prevent unwarranted monopolization by a single species. In the past, success of a similar approach – where a mouse infected with the *C. difficile* strain recovered after having a phylogenetically diverse mixture of bacteria injected in its gut – propelled research in this direction.[39] The authors rationalized that the resolution of dysbiosis is inherently what leads to the improved condition. Further proof-of-principle studies were conducted in humans before the technique gained acceptance.[40,41]

After screening and processing stool from healthy patients, the feces are transplanted into the patient's diseased gastrointestinal tract. The current standard protocol, agreed upon by leading experts in the field, includes a stringent screening process for the donor. This includes blood and stool testing for infectious agents such as human immunodeficiency virus (HIV), illicit drugs, immunosuppressive medications and allergens, amidst other biological factors.[34,42] After passing scrutiny, the donor stool is dissolved in milk and administered either through the upper-gastrointestinal route (e.g., through nasogastric tube, jejunal catheter, etc.) or the lower gastrointestinal route (e.g., colonoscopy).[34,43] While the procedures for the former route of administration are relatively cheaper and pose a lower risk, they often do not reach distal regions of the colon. The preferred method in current treatment is colonoscopy, with a success rate of over 89% for FMT.[43]

FMT is deemed successful in reported studies with rapid resolution of symptoms anywhere between two to three days up to six months and no associated complications.[40,42]. Whereas the *success* is defined by the resolution of diarrhoea/fever or a drop in white blood cell count, *failure* is signalled by recurrence.[42,43] Multiple clinical trials show success of the procedure based on the criteria above with few cases being irresponsive to treatment.[41–46] Recently in 2013, a clinical trial study stated that an initial treatment with vancomycin followed by a duodenal infusion led to such an overwhelmingly high success rate of 81% that the trial was terminated.[46] Some studies went a step further to characterize the gut bacterial species post-treatment using microarray hybridization and high throughput sequencing techniques. They validated that the microbial population indeed was diversified using the Simpson's Reciprocal Index for diversity of microbiota.[46,47] Increased diversification meant Bacteriodetes species (non-spore forming, anaerobic gram negative bacteria) increased while Proteobacterium species (including many pathogenic bacteria) decreased, the microbiota composition of patients essentially becoming indistinguishable from that of donors.[46,47] Furthermore, FMT (via colonoscopy) for initial treatment for RCDI was determined to be the most cost-effective strategy over competing drug therapies involving vancomycin and metronidazole.[48] To further increase the treatment's efficacy, risks of techniques used for delivery such as colonoscopy need to be evaluated, long-term follow up patient registries need to be established, and patient-centered procedures still need to be enacted.[8,49]

While current research in the domain is dedicated towards antimicrobial, toxin-binding or immune system modulating agents in clinical trials, it becomes incumbent to shed light on FMT as an alternative.[50] A cost-effective treatment that can be easily performed in a community hospital with minimal risks needs to be considered. While once both physicians and patients shied away from the procedure due to the *yuck factor*, it is now in the limelight as an alternative treatment for other diseases such as IBD as well.[51] Moreover, as the administration protocols for FMT are further standardized and tested to reduce costs and increase efficacy,[48] it is sure to become a routine procedure for an array of disruptive gastrointestinal illnesses.

Acknowledgements

I am grateful to Sr. Samina Shafique for cultivating the pursuit of scientific inquiry in me, to Drs. Sapna Sharma and Jean-Peal Paluzzi for sowing its passion and to Dr. Kevin Truong for harvesting its gains.

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