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Research Strategy

1. Significance

1.1 *Clostridium difficile*-Infection: A Major Health Epidemic

Clostridium difficile-infection (CDI) includes a wide range of illnesses, from uncomplicated diarrhea to toxic megacolon and multi-organ dysfunction syndrome necessitating colectomy (1). It is the most common cause of infectious nosocomial diarrhea in developed countries, and is now the single most common pathogen found among Healthcare-Associated Infections (HAI), accounting for 12% of all HAIs (2). In 2013, the Centers for Disease Control and Prevention (CDC) declared CDI one of only three urgent threats which they define as immediate public health threats that require urgent and aggressive action (3). The mortality rate from the severe forms of CDI is high and rising. The CDC estimated there were 250,000 hospitalizations and 14,000 deaths related to CDI in the United States in 2007 (12). A more recent CDC study raised the estimates to 453,000 hospitalizations and 29,000 deaths annually based on 2011 data (4). These two studies are in line with data from the Healthcare Cost and Utilization Project, which found that there were 336,600 hospitalizations and 30,600 deaths annually associated with CDI based on 2009 data (13). Despite these already staggeringly high statistics, the incidence and mortality rates from CDI continue to rise (5). The elderly are disproportionately affected by CDI. Hospital patients ≥65 have 5-fold higher rates of CDI than patients 45-64 and 20-fold higher than patients 18-45 years of age (6).

CDI is caused by *C. difficile,* a spore-forming, anaerobic, gram-positive bacillus that can be found as a colonizer in the colon of otherwise healthy adults. Some strains of *C. difficile* produce toxins (A and B), which are directly responsible for the pathogenicity of the organism; strains that do not produce toxins do not cause CDI (14, 15). CDI results from proliferation of toxigenic *C. difficile* that is no longer held in balance by the beneficial normal flora of the gut. Antimicrobial therapy that disrupts the normal intestinal flora is the single largest risk factor for CDI. Spread of *C. difficile* occurs via the fecal-oral route, where the organism enters the gastrointestinal tract by ingestion of spores from contaminated surfaces (16). Transmission occurs primarily via contact with skin contaminated by spores or from a contaminated environment (17, 18). The rising incidence of CDI has been attributed to the frequent use of broad-spectrum antibiotics, such as ampicillin, amoxicillin, clindamycin, fluoroquinolones, and cephalosporins (7, 8). The **current roadblock in the field of CDI** is that there is no established intervention to significantly reduce the incidence and mortality rates for this disease at the level of the individual patients who receive antibiotics. **The goal of this study is to prove that prophylaxis of high-risk patients is an effective means of preventing CDI.**

1.2 Emergence of Hypervirulent *C. difficile*: Implications on Increased Disease Severity

The hypervirulent, epidemic strain of *C. difficile*, NAP1/BI/027, is responsible for increased nosocomial outbreaks and concomitant morbidity and mortality worldwide (19-21). The NAP1/BI/027 strain is more resistant to fluoroquinolones than prior strains (22), and multiple reports cite that this epidemic strain is more difficult to treat and eradicate. Furthermore, this strain specifically has a lower susceptibility to metronidazole, which had previously been a cornerstone of treatment for *C. difficile*. The NAP1/BI/027 strain has variations in its toxin genes and produces increased levels of toxins A and B, as well as an extra toxin, known as the binary toxin, which accounts for its increased pathogenicity (19). This strain of the organism is a cause of worldwide outbreaks of CDI, and has been associated with significant increases in incidence, severity, and mortality (6, 19, 23). When examined at our institution, 60% of CDI was due to the hypervirulent strain of *C. difficile*, and was associated with higher rates of treatment failure and mortality (24).

1.3 Economic Impact of CDI

The economic burden associated with *C. difficile* infections is increasing. Current estimates of annual costs attributed to CDI vary, but are all higher than one billion dollars per year (12). Each episode of CDI increases both direct healthcare cost and length of stay for patients affected (25). With the emergence of the hypervirulent epidemic strain, and the associated increase in incidence and severity of CDI, along with more refractory and recurrent infections, estimates are that direct healthcare costs related to CDI may double over the next several decades. Several published studies have reported estimated annual overall costs of CDI in the United States and Europe that range as high as $4.8 billion based purely on hospital admissions (12, 26-29). In all of the studies, the investigators stated that their estimates were conservative and likely underestimated the total expense of treating CDI. The economic burden of CDI is even more significant as these studies only

consider direct healthcare costs and no study to date has attempted to ascertain the economic burden to the infected individual in terms of personal expenditures, missed workdays, poor quality of life, and decreased economic productivity (30). Establishing a standard-of-care model that reduces the incidence and mortality of CDI would have a major global impact on reducing annual costs related to CDI.

1.4 Laboratory Testing for CDI

Stool culture with cytotoxin neutralization assay is considered the most accurate test for the presence of toxigenic *C. difficile*; however, the test is extremely time-consuming and few laboratories perform these tests routinely (16, 22, 31). Enzyme immunoassay (EIA) can be used to detect the presence of toxin A and toxin B. This test is inexpensive, easy to run, and rapid, but lacks sensitivity (16, 22, 31). There is evidence that a positive EIA is more predictive of significant CDI (32). Commercial polymerase chain reaction (PCR) assays directly detect the presence of the toxin B gene (*tcdB*) in stool. Toxin B has been found in nearly all cases of CDI, and the molecular assay for the *tcdB* is highly sensitive for detection of strains of *C. difficile* associated with CDI (33). In several studies, sensitivities for PCR-based *C. difficile* assays were in the range of 83.6%-96.4% compared to 66%-67.2% for the EIA for toxins A and B (33-36). PCR-based assays represent a significant improvement in laboratory testing for *C. difficile*, but the inherent sensitivity of the test makes diagnosis of CDI more difficult, since it will easily detect colonization and could lead to over diagnosis of CDI (32). *C. difficile* colonization can be easily detected using PCR testing in formed stools or using swabs (37, 38). At our institution detection rates doubled when our standard testing modality changed from EIA to PCR.

1.5 Asymptomatic Colonization: Role in CDI

1.5.1 Overall Rate of Asymptomatic Colonization

Numerous studies have looked at the rate of asymptomatic colonization with *C. difficile*. The estimates in hospitalized patients vary from 4%-21% (11, 39-51), and not all studies separate colonization with toxin-producing strains from non-toxigenic strains, or assay patients early in their admission to the hospital. Studies of patients in long-term care or rehabilitation facilities show even higher levels of colonization from 17%-51% (52, 53). In healthy patients outside the healthcare environment, studies show overall colonization rates from 6.6%-11%, with toxigenic colonization rates from 4.3%-8.4% (54-57). The newer studies show higher rates of colonization in hospitalized patients, which is easily explained by changes to more sensitive detection methods and the epidemiology of *C. difficile*. The most recent estimates of colonization with **toxigenic *C. difficile* at the time of admission** to the hospital range from 8%-15% (11, 39, 42, 43, 49).

1.5.2 Is There a Protective Effect of Asymptomatic Colonization with C. difficile?

There has been debate in the clinical community of the role colonization with *C. difficile* plays in the risk of developing CDI. A meta-analysis by Shim et al. (58) performed in 1998 stated that non-colonized patients were four times more likely to develop CDI than colonized patients, but could not explain the mechanism behind the apparent protection. Kyne et al. (48) performed a study in 2000 to prospectively examine the immune response to colonization. This study found that newly acquired colonization with *C. difficile* did not progress to CDI if the patients had high levels of **pre-existing IgG against toxin A**, which they went on to suggest was the mechanism behind Shin's findings. This paper has historically been cited in the CDI field as demonstrating that colonization with *C. difficile* is protective against developing CDI. However, due to the prospective design of Kyne's study, patients who were already colonized on admission to the hospital were dropped from analysis. The paper did not truly conclude that colonization was protective; instead, the authors stated that patients with IgG against toxin A were protected from progressing from newly established colonization to infection. The protective effect of antibodies against *C. difficile* toxin has been demonstrated in several clinical trials using monoclonal antibodies against *C. difficile* toxins to prevent recurrence of CDI (59-61). Kyne's paper did not provide commentary on whether pre-existing colonization influenced development of CDI. Shin's meta-analysis has recently been challenged by Zacharioudakis et al. (11) as it did not separate whether the protection was based on colonization with toxigenic or non-toxigenic *C. difficile*, and two of the four studies used in the meta-analysis did not assay for colonization at the time of admission to the hospital. Shin's study was performed prior to the emergence of the hypervirulent strain and thus may not be reflective of current issues.

More recently, it has been made clear in both an animal model (62-64) and a clinical trial (65) that **it is colonization with non-toxigenic *C. difficile* that is protective against CDI**. The proposed mechanism is that the non-toxigenic *C. difficile* inhabits the same ecological niche in the colon and prevents establishment of the toxigenic strains by outcompeting them.

1.5.3 Asymptomatic Toxigenic C. difficile Colonization is Associated with CDI Development

Recent studies have looked specifically at colonization with toxigenic *C. difficile* as a risk for CDI. In a systematic review and meta-analysis, Zacharioudakis and colleagues identified that 8% of patients at the time of hospital admission (10% in North America) were carriers of toxigenic *C. difficile* and were six times more likely to develop CDI (21.8%) than non-carriers, indicating the predominant role toxigenic colonization plays in development of CDI (11). It is thought that colonization becomes a problem to the carrier after they are treated with antibiotics when the asymptomatic state can blossom into CDI (22). Riggs et al. found that 20% of patients in long-term care facilities who were colonized with toxigenic *C. difficile* developed CDI during follow up (53) and Merciniak et al. found 44% of patients in rehabilitation units colonized with toxigenic *C. difficile* developed CDI during follow up (52). Neither of these studies were limited to patients who received antibiotics. Hung and colleagues prospectively demonstrated that patients with toxigenic *C. difficile* colonization had a four times higher increased risk for developing CDI than patients admitted who were not colonized (66), while Gupta et al. conducted a large multicenter prospective trial of the epidemiology of hospital-acquired *C. difficile* colonization and CDI, and found that patients with asymptomatic toxigenic *C. difficile* colonization were 11.7 times more likely to develop CDI (9) and 45% of hospital onset cases of CDI were in colonized patients. Tschudin et al. found pre-existing colonization with toxigenic *C. difficile* conferred a 10.29 relative risk for developing CDI (10).

Epidemiologic investigations using molecular techniques have demonstrated that many new hospital-onset cases of CDI are not attributable to transmission from known cases of CDI in the hospital (42, 67-71). Prior prevailing thought was that CDI was preceded immediately by acquisition of *C. difficile* from other hospitalized patients when the patient's colon was rendered susceptible to infection with *C. difficile* due to antibiotics altering the protective flora. This data illustrates the likelihood that prior colonization with toxigenic *C. difficile* is a major risk factor for CDI. In addition, in studies using molecular typing, relapse of infection with the same strain of *C. difficile* has been shown to occur out as far as 26 weeks, long past what is generally considered the limit for relapse of CDI (8 weeks), also suggesting that persistent colonization with toxigenic *C. difficile* leads to increased risk of CDI (72, 73). The mean age of toxigenic colonization is ~58 (10, 39), disproportionately affecting older patients. Multiple factors associated with aging have been shown to increase this risk (74).

1.6 The Downfalls of Current Practices for Prevention of CDI

1.6.1 Infection Control

The conventional assumption is that patients with CDI and perhaps asymptomatic carriers are the major reservoirs for *C. difficile* and spread the infection within the hospital. Careful adherence to infection control policies is the status quo for the control of *C. difficile* at hospitals, nursing facilities, and long-term care and rehabilitation facilities (16, 75, 76). Standard practice in infection control places patients who have CDI in isolation or cohorts them with other CDI patients. Use of personal protective equipment and hand washing is required of all those who enter the room (16, 75). While infection control procedures have proven helpful, there is limited ability to assure compliance with isolation and hand hygiene procedures.

Ultimately, infection control procedures do not solve the problem of CDI. Eyre et al. found that while rare, spread from an asymptomatic carrier may be a risk factor for development of CDI due to the sheer number of cases of asymptomatic carriers (77). Walker et al. showed that in a setting with strict infection control measures, contact with patients found positive for *C. difficile* was not related to new cases of CDI. The authors found that 75% of new cases were not linked to a patient source. This indicates that 75% of the cases of CDI could not be stopped by isolating patients with active diarrhea due to *C. difficile* (70). In a separate study, Eyre et al. demonstrated that most cases of CDI presenting in the hospital are either genetically distinct from other known cases in the hospital or share no known epidemiologic contacts (68). Therefore, it is imperative that novel avenues are developed beyond basic infection control practices to combat the rising incidence of CDI.

1.6.2 Antibiotic Stewardship

Antibiotic stewardship in regard to *C. difficile* seeks to limit inappropriate use of antibiotics, such as those known to be more likely to disrupt colonic flora and induce CDI (75, 76). While this approach also has some success in decreasing rates of CDI, it is not possible to eliminate the use of high-risk antibiotics, as they are an important part of the antimicrobial armamentarium. **Stewardship efforts, while important, cannot reduce the risk of CDI in the face of decreasing effective therapies for other infections**.

1.7 The Need for New Approaches for Prevention of CDI

The emergence of the hypervirulent strain of *C. difficile* exacerbated an already significant problem to the point where it is now one of the three top infectious threats facing healthcare today. The cost in terms of both dollars and human lives is staggering. Current prevention strategies, while they help, are not enough to deter the ever-increasing problem of CDI. Colonization with toxigenic *C. difficile* has been shown to increase the risk of CDI in hospitalized patients at least six-fold, and the use of high-risk antibiotics is the single largest risk factor for CDI. Current molecular assays are sensitive enough to detect toxigenic *C. difficile* in asymptomatic patients with formed stools, and being able to detect those patients admitted to the hospital with significantly higher risk for CDI is the first step in being able to intervene and prevent it.

2. Innovation

2.1 A Novel Approach to Prevention of CDI in At-Risk Patients

The proposed study addresses AHRQ's Key Question #2 for *C. difficile*, "What are effective prevention strategies?" and has the ***potential to cause transformative change*** in the methodology of preventing CDI, and to alter the standard of care when using high-risk antibiotics in the hospital setting.

Multiple studies using sensitive molecular techniques have now shown that colonization with toxigenic *C. difficile* is a risk factor for developing CDI (9, 18, 22, 53, 66). Therefore, it is critical that innovative methods are established to provide efficient and long-term mechanisms to screen carriers of *C. difficile* to halt progression of CDI. We ***hypothesize*** that by determining which patients are colonized with toxigenic *C. difficile*, and therefore are at higher risk for developing CDI when treated with high-risk antibiotics, we can initiate an intervention to reduce that risk. The most effective, and affordable, intervention, we believe, is prophylactic treatment with vancomycin, which is efficacious against *C. difficile* (1). Our ***goal*** for this study is to implement a large-scale first-of-its-kind clinical trial to test our hypothesis by: 1) screening high-risk inpatients for the presence of toxigenic *C. difficile*; and 2) providing prophylactic treatment to prevent CDI by using vancomycin during the at-risk period when patients are treated with high-risk antibiotics known to induce CDI. In our opinion, the proposed research is ***innovative*** because it presents a significant departure from the current standard of care model for prevention of CDI, which is based on infection control and antibiotic stewardship. In this innovative model, highly sensitive PCR-based assays will be used to screen patient stool samples followed by strategic prophylactic treatment with vancomycin that will reduce *C. difficile* levels during the critical window when antibiotics have depressed the normal flora of the colon and the patient is at risk for onset of CDI. The inherent sensitivity of the PCR-based test makes it ideal for screening the asymptomatic population for presence of toxigenic *C. difficile* (33) even in formed stools (37, 38).

2.2 Primary Prophylaxis: The New Frontier in CDI Control

No studies to date have considered primary prophylaxis in the appropriate subgroup of patients to prevent CDI, despite the fact that prophylaxis is a universally accepted method of preventing the occurrence of infection in diverse populations of patients (78-81). The largest barrier to such studies is identifying the subgroup of patients who would benefit from prophylaxis. Many clinicians have taken to using secondary prophylaxis with either vancomycin or metronidazole in patients with a prior history of CDI to prevent recurrence. Several retrospective studies support this practice. Van Hise et al. reported a reduction in CDI recurrence from 35% to 1% using vancomycin prophylaxis (82). Wong and Riska presented similar findings with a reduction in recurrence rates from 20% to 7%, but included metronidazole as a prophylaxis agent (83). In a recent retrospective review of CDI cases, Drekonja et al. observed that patients who required other antibiotics during treatment for CDI had no increased risk for recurrence whereas patients who required other antibiotics after completing treatment for CDI had a significant increase in CDI recurrence (84). This indicates the potential role prophylactic treatment can play in reducing CDI cases if patients are treated in the optimal window. Rodriguez et al. performed a retrospective analysis of 12,000 patients at high risk for CDI they found patients who received incidental metronidazole for an indication other than CDI had an odds ratio of 0.21 for developing CDI compared to patients who did not receive metronidazole (85). Dupont, in an editorial of Rodriguez's study, described the concept of chemoprophylaxis against CDI as a potentially important concept, but raised concerns with use of metronidazole given its systemic absorption and potential side effects as well as its low efficacy against the hypervirulent strain (86). Dupont also discussed several other antibiotics with potential for use as prophylaxis against CDI, but most are highly expensive and would be difficult to justify. Oral vancomycin, however, is the most commonly used agent for treatment of CDI, and when compounded, as is common practice at most hospitals, would be affordable as prophylaxis. The concern was raised about use of

vancomycin for prophylaxis against CDI leading to an increase in colonization with vancomycin-resistant *Enterococcus* (VRE), which would need to be monitored. Another concern is the possibility that overuse of vancomycin will lead to resistance in *C. difficile*; however, despite high levels of use for the treatment of CDI, resistance to vancomycin remains essentially unseen (87-89). Dubberke's recent study on *C. difficile* colonization (43) also raised the point that several studies suggested that antimicrobial prophylaxis might prevent *C. difficile* colonization or CDI, but rightly suggested caution in using this as an approach to CDI prevention. Clinical trials would certainly be needed to prove that antimicrobial prophylaxis to prevent CDI is both effective and safe, which is the goal of this study.

Johnson et al. demonstrated that oral vancomycin was able to suppress *C. difficile* in asymptomatic *C. difficile* carriers in a randomized placebo controlled trial (34). Their study, though small, showed that 9 of 10 patients receiving vancomycin became culture-negative during and immediately after treatment, compared to 3 of 10 receiving metronidazole and 2 of 10 receiving a placebo. However, this decolonization was transient, and most patients became re-colonized within weeks. Thus, vancomycin appears to be able to suppress *C. difficile* in colonized patients, at least temporarily. **The goal of prophylaxis is not to eradicate the colonization but to suppress it at the critical time when the risk for CDI is greatest**. This makes vancomycin attractive as an option for primary prophylaxis against CDI.

It has been estimated that even a 5% decrease in the incidence of CDI could lead to a net cost savings in direct healthcare expenditures of $150 million a year (12, 28, 30). This savings would need to be balanced against the increased cost of screening and prophylaxis. The key is that while universal prophylaxis would be prohibitively expensive, prophylaxis based on screening for risk would likely bring about a significant decrease in healthcare expenditures as well as morbidity and mortality from CDI.

2.3 Clinical Trial to Prove Best Clinical Practice: Screening to Prophylax against CDI (SToP CDI)

There is an urgent need to develop a clinical protocol that will effectively reduce CDI incidence and mortality rates in patients at high risk for CDI. To date, no prospective studies have evaluated or identified preventative methods to ***limit the onset of CDI***. Probiotics have been suggested many times, but data in the literature is contradictory and various preparations are highly variable in both content and viability (90, 91).The ***objective*** of this proposed clinical study is to determine whether screening for toxigenic *C. difficile* colonization among admitted patients receiving high-risk antibiotics predicts whether these patients will develop CDI when compared to non-colonized patients (Specific Aim 1), and if treating those patients prophylactically with vancomycin will decrease CDI incidence, associated symptoms, and mortality rates (Specific Aim 2). Our expected outcome is that prophylaxis with vancomycin will significantly reduce the rate of CDI in patients who are carriers of toxigenic *C. difficile* and are exposed to high-risk antibiotics.

We believe this contribution will be ***significant*** because it has the potential to reverse the increasing trend associated with CDI incidence and mortality rates, decrease the economic burden associated with the disease, and improve patient quality of life. If our hypothesis is confirmed, screening for and prophylaxis of carriers of toxigenic *C. difficile* with vancomycin will be cost effective and can be easily implemented as routine care with minimal discomfort and adverse impact to patients. This method has the potential to revolutionize the current standard of care.

3. Approach

Specific Aims

Aim 1: Determine the prevalence of toxigenic *C. difficile* colonization among the inpatient population and the incidence of CDI in patients treated with high-risk antibiotics with respect to their state of colonization with toxigenic *C. difficile*.

1.1 Assay inpatients initiated on high-risk antibiotics for the presence of toxigenic *C. difficile* in their stool.

1.2 Determine the incidence of CDI in toxigenic *C. difficile*-positive patients treated with high-risk antibiotics.

1.3 Determine the incidence of CDI in toxigenic *C. difficile*-negative patients treated with high-risk antibiotics.

Aim *2*: Determine the effect of vancomycin prophylaxis on patients receiving high-risk antibiotics who are colonized with toxigenic *C. difficile* based on molecular testing.

2.1 Determine the incidence of CDI in this subgroup of patients.

2.2 Determine the severity and outcome of CDI in this subgroup of patients.

3.1 Research Design

3.1.1 Clinical Study Overview

Beaumont Health is an eight-hospital regional health care system with a total of 3,337 beds, 35,000 employees and 5,000 physicians in Southeast Michigan. Beaumont Hospital – Royal Oak is a 1,070-bed, major academic, research, and referral center with Level I trauma designation. In 2014 there were 58,539 admissions and 48,387 surgeries, among the highest in the United States. In 2015, 13,812 *C. difficile* PCR tests were run at our institution. Our infectious diseases clinical trials unit runs numerous clinical trials at the Beaumont – Royal Oak. We have the necessary population and tools in place to make this study a success.

Screening to Prophylax against CDI (SToP CDI) will be a prospective, multi-center, double-blinded, randomized, placebo-controlled study of the effectiveness of vancomycin vs. placebo for preventing CDI in patients colonized with toxigenic *C. difficile* and receiving high-risk antibiotics (**Figure 1**). We plan to enroll 2500 inpatients from Beaumont for the initial screening. Based on Zacharioudakis et al. (11) we expect 8% of the screened patients to be positive for *C. difficile* colonization. Colonized participants will be randomized to one of two arms with 100 subjects per arm. We plan to evaluate all subjects for development, severity, and outcome of CDI. Patients who are initiated on high-risk antibiotics for a minimum expected duration of three days will be identified via an automated search of the Electronic Medical Record (EMR), which has already been built and is currently used to identify potential patients for clinical trials. After initial screening for inclusion and exclusion criteria, the patients will be approached to participate in the study. Written informed consent will be obtained from all study participants. Consented patients will have a stool sample collected and tested for presence of toxigenic *C. difficile* by PCR using the BD Max assay (Becton Dickinson), which is used for routine testing for *C. difficile* at our institution. Patients who test negative (are not colonized with toxigenic *C. difficile*) will simply be followed for development, severity and outcome of CDI. Patients who test positive (are colonized with *C. difficile*) will be randomized to one of two arms:

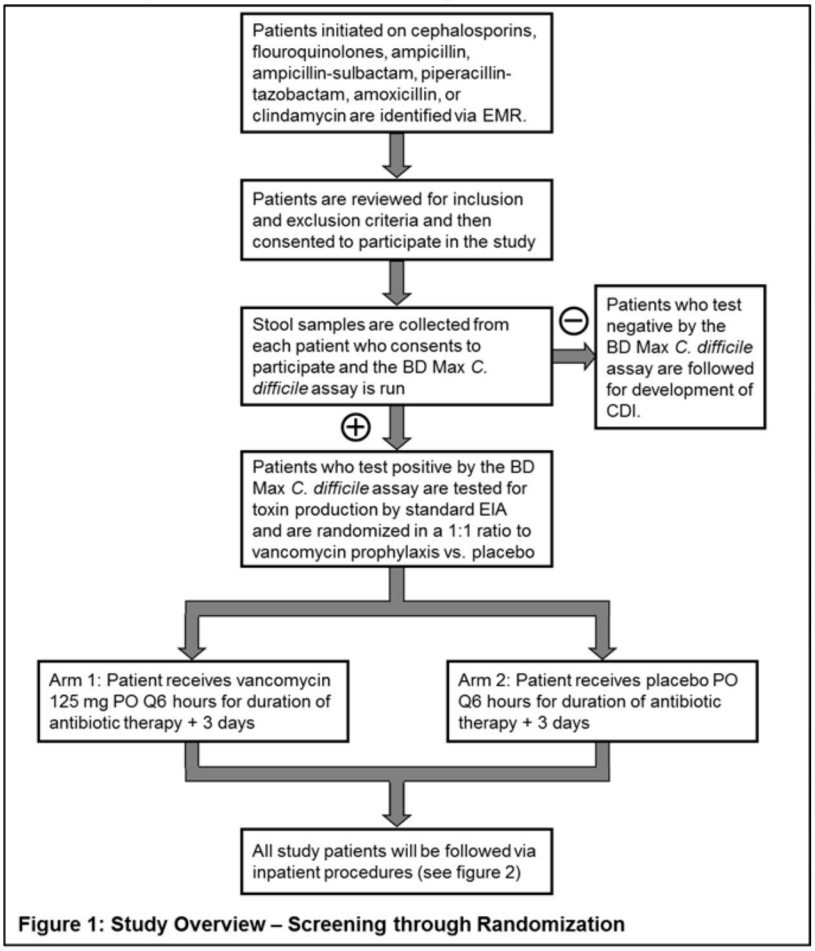

Figure 1: Study Overview – Screening through Randomization

Arm 1: Patients receive 125 mg vancomycin PO q6 hours as prophylaxis against *C. difficile* for the duration of their antibiotic treatment +3 days.

Arm 2: Patients receive placebo PO q6 hours for the duration of their antibiotic treatment +3 days (serves as the control group).

3.1.2 Preliminary Data

Beaumont possesses the patient volume as well as the necessary clinical and research expertise to carry out the SToP CDI study. Our research group has a successful history of high recruitment and specifically high recruitment into *C. difficile* trials. Recruitment feasibility of patients who develop CDI after admission to our institution was determined from the previous three years of epidemiologic data for hospital-onset cases of CDI within our institution (**Table 1**). The National Healthcare Safety Network defines a case of hospital-onset CDI as any positive laboratory test for *C. difficile* which occurs >72 hours after admission. This will reflect a certain amount of colonization; as such, our Department of Epidemiology reviews all cases of laboratory-identified hospital-onset CDI via the EMR to attempt to limit

Table 1: Rates of Hospital Acquired *C. difficile* and CDI

Year	LAB-ID cases of *C. difficile*	Patient Days	Rate per 1000 patient days	Clinical CDI per EMR Review	Patient Days	Rate per 1000 patient days
2013	338	311608	1.085	61	334999	0.182
2014	330	316979	1.041	77	339292	0.227
2015	229	229255	0.999	44	258584	0.170

Note 2015 Data is January through September only

this to clinical cases. They define a case as any non-relapsed episode of CDI occurring >72 hours after admission and during the admission or within 30 days of discharge in patients without another potential cause of diarrhea. The actual number of hospital-onset cases likely lies somewhere in between, as many discharged patients who develop CDI as an outpatient may be missed and some of the cases felt to have an alternative explanation from review of the EMR may actually have CDI.

We currently have a search engine built within the EPIC EMR at our institution that finds all patients ≥18 years of age who are started within a preset time on high-risk antibiotics for CDI. All study personnel have the capability of accessing this search. On a typical day, this search will identify 50-200 patients initiated on antibiotics. Day-to-day repeat patients are noted, and the actual number of patients suitable for recruitment is expected to be closer to 50 per day than 200 per day.

A previous prospective observational study at our institution determined that the hypervirulent strain was present in 60% of cases of CDI (24). Given the nature of the hypervirulent strain, we expect that our estimate of the percentage of patients who are carriers of toxigenic *C. difficile* is likely accurate or even low.

3.1.3 Patient Recruitment

Dr. Sims has served as PI on numerous multicenter phase II, phase III, phase IV, and registry trials. He was part of the design team for the Discover Registry for Fidaxomicin use. In addition to the investigators, a fulltime nurse coordinator a fulltime research coordinator, as well as several volunteers, will be 100% dedicated to help conduct this clinical trial. The nurse coordinator for this trial has been lead coordinator on all of the interventional CDI trials run by Dr. Sims. Our team has a record of high recruitment for these trials and, given the nature of this trial, we estimate that the recruitment success rate for this study will be at least 10% of patients approached. **In order to complete study enrollment in four years, an average of 1.7 patients per day would need to be enrolled, recruiting an average of 3 patients per day will complete enrollment in just over 2 years and 3 months**. Subjects will be identified through review of the EMR, which will automatically narrow the search to patients in the appropriate age range (both female and male patients), and exclude patients using antibiotics with activity against *C. difficile*. The inclusion and exclusion criteria for the study are listed in **Table 2**.

Review will be performed by the study coordinator with additional review of any subjects with unclear eligibility criteria by Dr. Sims or one of the sub-investigators when he is not available. The eligible patients will be approached for participation in the study by a study coordinator or a physician member of the study team. The purpose and basic requirements of the study will be explained, the patient will be given the opportunity to ask questions regarding the trial, and can choose to speak with one of the physicians if they desire. Subjects who meet inclusion and exclusion criteria and express interest will be given the informed consent document to review. The background, purpose, benefits, and risks of the trial will be explained to the patient, who will then be invited to ask questions to their satisfaction. The cost of the study drug and any study procedures will be covered by the study with no expense to the patient. The patient must provide written informed consent prior to enrollment. Failure to provide written informed consent will render the patient ineligible to participate in the trial.

Table 2: Inclusion-Exclusion Criteria for the SToP CDI Study

Inclusion Criteria (must meet ALL)	Exclusion Criteria (cannot have ANY)
Expected duration of admission sufficient to complete screening and enrollment or willing to have a sample brought in for testing should they be discharged before screening is complete	Inflammatory bowel disease or chronic diarrhea
Age ≥18	CDI within prior 90 days
Able to give informed consent	Current diarrhea
Initiated on one of the following antibiotics within the prior 72 hours with an expected duration of at least 72 hours from enrollment: clindamycin, ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanate, fluoroquinolones, carbapenems, piperacillin/tazobactam, or any cephalosporin	Currently on metronidazole, oral vancomycin, rifaxamin, fidaxomicin, or any other antibiotic active against *C. difficile*
Maximum expected duration of antibiotics 8 weeks	Known allergy to vancomycin
Able to take oral study medications	Pregnancy or breast feeding (determined prior to randomization)
Able to provide a stool sample	Life expectancy of less than 60 days
Reasonably expected to be able to complete follow up	Current ileostomy, colostomy or other form of surgically disconnected gut
	Travel to an area of endemic diarrheal illness within the last 30 days
	Participation with other research trials that could impact the results of this trial within the last 30 days
	Previously enrolled in this study

3.1.4 Study Procedure

A. Patient Baseline Data

All patients enrolled in the study will have the following data recorded using a standardized data collection form: 1) admission diagnosis; 2) diagnosis leading to initiation of antibiotics; 3) medication record; 4) medical and surgical history; 5) present status of bowel movements: number per day and consistency; 6) vital signs; 7) white blood cell count (WBC) and differential; 8) baseline and current serum creatinine; 9) other laboratory indicators of systemic disease if available (i.e., C-reactive protein, procalcitonin); 10) current abdominal pain, nausea, vomiting, or abdominal bloating; 11) allergies; 12) demographic information, including age, race, sex; 13) social information, including marital status, place of residence, and who they reside with; and 14) contact information, the best phone number to reach them at, and the best times they can be reached.

B. Interventions

The following interventions will be administered to the randomized patients in the following study arms:

Arm 1: Vancomycin 125 mg PO q6 hours (using compounded liquid vancomycin)

The patient will take vancomycin 125 mg PO q6 hours as prophylaxis against CDI from enrollment until 72 hours after completing their planned course of antibiotics. The rationale behind this arm is that the vancomycin will suppress the overgrowth of *C. difficile* required to develop CDI.

Arm 2: Placebo PO q6 hours

The patient will take a placebo PO q6 hours from enrollment until 72 hours after completing their planned course of antibiotics. Vitamins will be added in order to confer a bitter taste and preserve the blind.

C. Laboratory Evaluations

Immediately after randomization, stool from all randomized patients will be tested for: the presence of the hypervirulent strain using the Xpert *C. difficile*/Epi test (Cepheid); detectable production of toxins A and B (not simply presence of the *tcdB* gene) by EIA (*C. difficile* Premier toxins A and B; Meridian Biosciences); and pre-existing colonization with VRE by stool culture. In addition, a single blood draw, timed with routine labs, will be drawn to assay for pre-existing antibodies to the *C. difficile* toxins (Quest Diagnostics). Patients will be given the option to have stool frozen for possible future studies of *C. difficile* including possible microbiome analysis.

D. Inpatient Follow Up

During the inpatient period, the subject will be evaluated daily via the EMR and seen at a minimum weekly by study personnel (study coordinator, research assistant, or study physician) (**Figure 2**). Upon each evaluation they will have the following data recorded: 1) present status of bowel movements: number per day and consistency; 2) vital signs (including maximum temperature and minimum blood pressure); 3) available laboratory data (including WBC and serum creatinine); 4) any current abdominal pain, nausea, vomiting, or abdominal bloating; 5) any signs of adverse reaction to the study medication; 6) changes in medications; 7) planned date of discharge; and 8) compliance with study medication. If at any time a subject shows signs or symptoms of CDI, particularly at least 3 episodes of diarrhea in a 24-hour period for 2 days in a row, they will be tested for *C. difficile* and evaluated by Dr. Sims, or one of the physician sub-investigators, to determine if a clinical diagnosis of CDI exists. If diagnosed with CDI, the patient will be treated per standard of care. Any evaluation and treatment for CDI will not be covered by this study as it is under standard of care. If their stool is positive for *C. difficile* by PCR, it will be tested by EIA for toxin production and the EIA will be covered

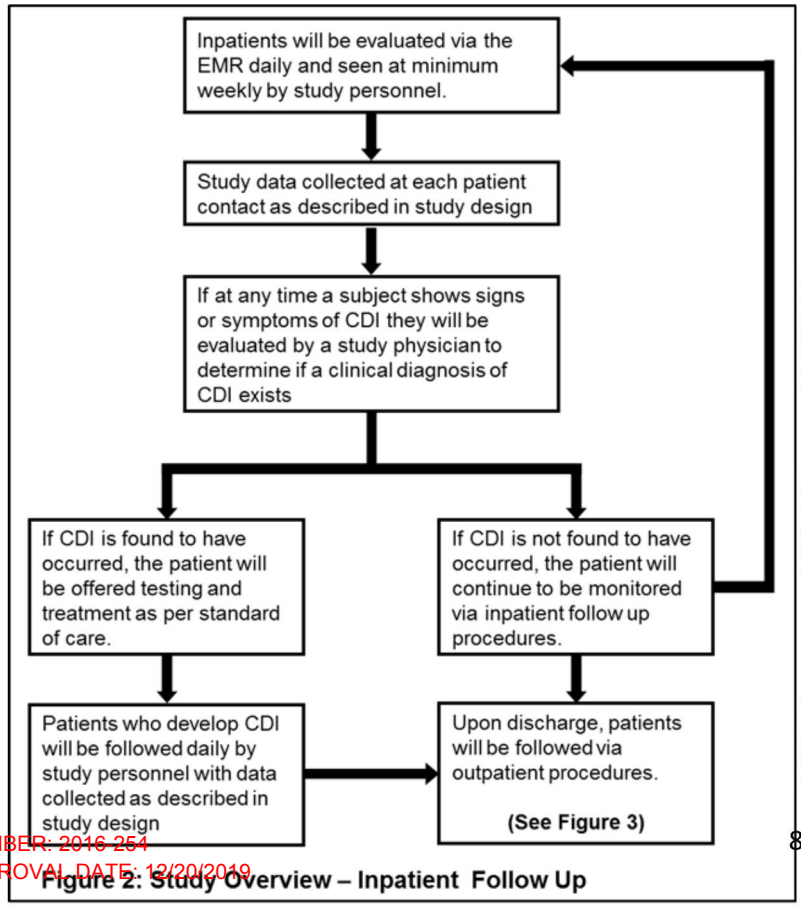

Figure 2: Study Overview – Inpatient Follow Up

by the study, as it is not standard of care. In the case of patients who were already *C. difficile* toxin-positive by EIA at randomization, the PI will rely on continued production of toxin as ascertained by EIA and clinical judgment based on symptoms.

During an episode of CDI, the following information will be collected daily: 1) general clinical status; 2) present status of bowel movements: number per day and consistency; 3) maximum temperature in the last 24 hours; 4) presence of hypotension; 5) available laboratory data (including WBC and serum creatinine); 6) any current abdominal pain, nausea, vomiting, or abdominal bloating; 7) current treatment regimen; 8) imaging studies of the colon, if available; 9) evidence of toxic megacolon, pancolitis, ileus, or intestinal perforation; 10) colonoscopy results, if performed; and 11) need for, and results of, any surgery for treatment of CDI.

E. Outpatient Follow Up

Patients who tested negative for colonization with *C. difficile* and were not randomized to a treatment arm will be followed for 12 weeks after stopping antibiotics. These patients will receive a phone call 12 weeks after completing antibiotic treatment. Patients who were randomized to a treatment arm will be followed weekly while on the study medication and for 12 weeks after completion of the study medication. The randomized patients will receive phone calls at the end of treatment, 4 weeks post treatment completion, 8 weeks post treatment completion, and at 12 weeks post treatment completion. The exact duration of time for which use of antibiotics places one at risk for CDI is unclear. Various studies have shown the risk to continue anywhere from 4 to 26 weeks (31, 72, 73, 92). We reviewed recent CDI trials on clinicaltrials.gov and found they follow for recurrence from 4 to 12 weeks, we elected to use the longest follow-up period of 12 weeks for this study. All patients enrolled in the study will be followed for 12 weeks by a phone call (**Figure 3**). At each contact, all patients will be asked the following questions:

1) What is the present status of your bowel movements: number per day and consistency?
2) Have you had a fever? If so, how high?
3) Do you have any abdominal pain, nausea, vomiting, or abdominal bloating?
4) Have you been readmitted to the hospital since discharge from the hospital?
5) Have you started any antibiotics since discharge from the hospital?
6) Have you been diagnosed with CDI since discharge from the hospital?
7) Have you experienced any other changes to your heath since discharge from the hospital?

The following additional questions will be asked of randomized patients who go home on study medications only, until medications are complete (a diary form will be supplied for them to record each dose taken at home and any noted side effects):

8) Have you missed any doses of the study medication and if so which?
9) Have you had any adverse reaction to the study medication?

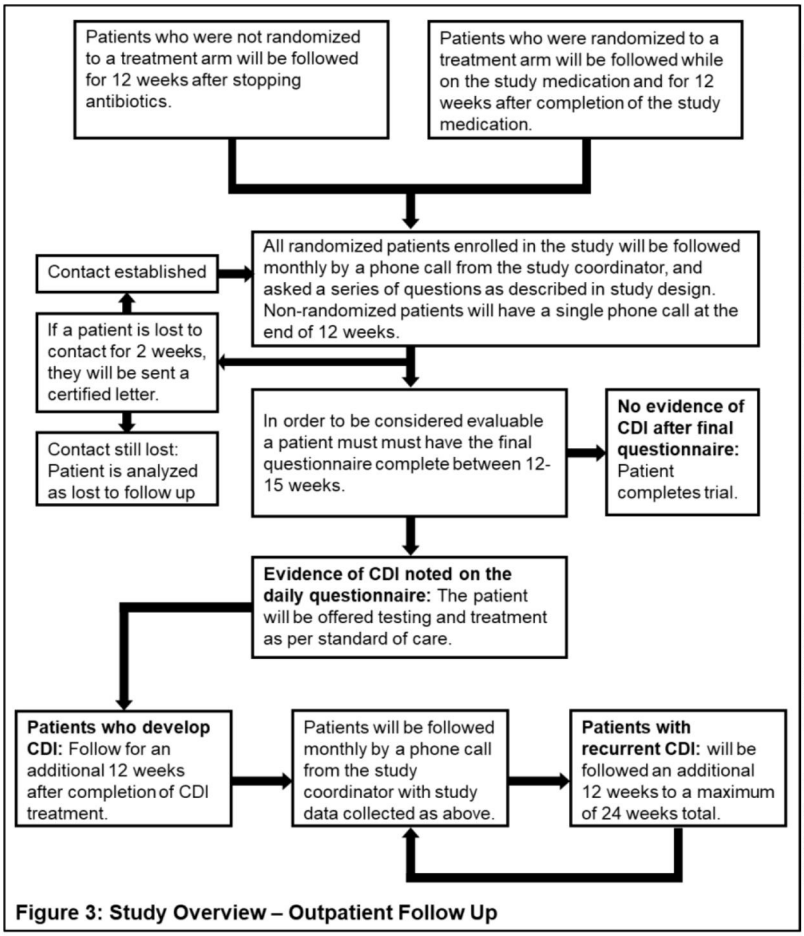

Figure 3: Study Overview – Outpatient Follow Up

At the end of the study medication and at the end of follow up, all randomized patients in both arms will be asked to submit a stool sample for retesting for *C. difficile* colonization and colonization with VRE to determine the rate of clearance of toxigenic *C. difficile* colonization and the rate of acquisition of VRE based on the intervention.

In order for a randomized patient to be considered fully evaluable, they must submit the end of treatment stool sample, respond to the questions at the time of the phone call monthly, and have completed the final phone call between 12 and 15 weeks after study treatment ended. If a patient misses their monthly

phone call, and we cannot contact them by phone for more than a week, they will be contacted by certified letter. If any patient is suspected of having CDI based on the phone call, they will be called in for evaluation by Dr. Sims or one of the physician sub-investigators, to determine if a clinical diagnosis of CDI exists. They will be offered testing and treatment as per standard of care if CDI is found to have occurred. Any evaluation and treatment for CDI will not be covered by this study as it is under standard of care.

Patients with CDI

Patients who develop CDI will be treated as per standard of care by their physicians. They will be followed as an outpatient for an additional 12 weeks after resolution of symptoms. They will be contacted by phone at 16 weeks, 20 weeks and 24 weeks after the initial 12 week follow up has ceased. The following information will be obtained from the patient: 1) general clinical status; 2) present status of bowel movements: number per day and consistency; 3) any fever in the last 24 hours; 4) any current abdominal pain, nausea, vomiting, or abdominal bloating; 5) current treatment regimen; 6) readmission to the hospital since last discharge; 7) initiation of antibiotic use since discharge from the hospital; and 8) diagnosis of CDI recurrence since discharge from the hospital. All relevant laboratory studies available through the EMR (including EPIC Everywhere to access data from other institutions that use EPIC) will be collected. Patients with recurrent CDI will be followed an additional 12 weeks out to 24 weeks at weeks 16, 20 and 24 by phone, to observe the natural history of their recurrences.

3.1.5 Study Endpoints

The *primary endpoint* of the study is development of CDI as ascertained by clinical presentation and laboratory testing. The *secondary endpoints* of the study include the following: 1) Severity of CDI as measured by clinical presentation and laboratories based on current Infectious Diseases Society of America (IDSA) guidelines; 2) Rate of conversion from colonization to CDI as related to presence of *C. difficile* toxin IgG; and 3) Rate of colonization with VRE after prophylaxis with vancomycin.

The IDSA guidelines define severity as follows: mild CDI is defined as diarrhea alone; moderate CDI is defined as diarrhea in combination with associated signs or symptoms of system toxicity but not meeting the definition for severe CDI; severe CDI is defined by the presence of a WBC ≥15,000 cells/μL and/or a creatinine ≥1.5 times the premorbid level; and severe complicated CDI shows hypotension, shock, toxic megacolon, or ileus (58). We recently performed a review of CDI severity at our institution and found 54.6% mild/moderate, 35.8% severe, and 9.6% severe complicated (93).

The outcome of treatment for CDI definitions includes the following: 1) cure is defined as complete resolution of symptoms at the end of follow up; 2) treatment failure is defined as requiring a change in therapy due to inadequate response; and 3) relapse is defined as a return of symptoms within 12 weeks after cessation of therapy.

3.1.6 Study Timeline

We propose a 5-year clinical trial. Recruitment will begin immediately after grant award and final IRB approval is obtained, and is expected to continue for just under four years. The intent is for the first patient to be recruited at start of Quarter 2, Year 1. At an average of 2 patients recruited per day, which we feel is reasonable, and allowing an extra six months for potential unforeseen circumstances or slow recruitment, we plan for recruitment to be complete by the end of Quarter 1, Year 5. Given the nature of the follow up, and based on experience with questionnaire-based studies, we estimate the dropout rate will be at most 10%. The 200 randomized subjects should provide at least 180 evaluable subjects. We will benchmark our progress based on the number of patients recruited, number of patients who complete the follow up, number of samples collected, total cases of CDI, and positive VRE cultures in the patient population. Follow up of patients in the study is expected to be completed by the end of Quarter 2, Year 5, with the possibility that a small number of subjects with recurrent CDI may need to be followed for up to an additional Quarter. Data analysis and manuscript preparation will begin at the start of Quarter 3, year 5, and will conclude by the end of Year 5

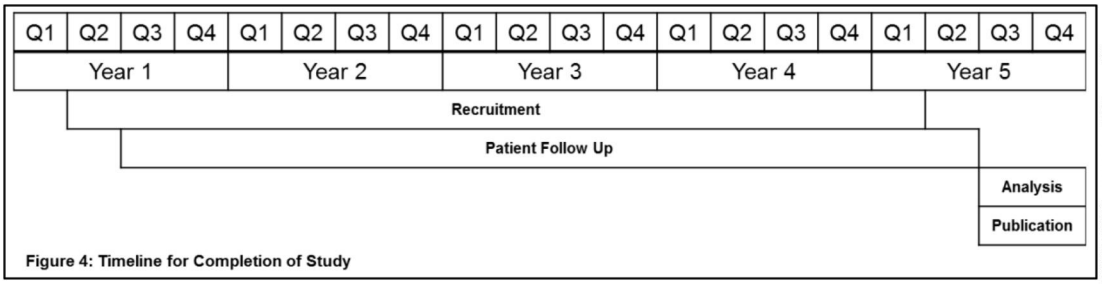

Figure 4: Timeline for Completion of Study

(**Figure 4**).

3.2 Statistical Analysis

3.2.1 Sample Size Calculation

The current rate of asymptomatic toxigenic *C. difficile* carriers in the hospitalized population is estimated to be a minimum of 8% based on multiple studies (11, 39, 42, 43, 49). We plan to obtain consent from 2500 patients, of which 8% would yield 200 subjects to randomize.

For the purposes of this sample size estimation, we began our calculation based on the assumption that 50% of patients colonized with toxigenic *C. difficile* will convert to CDI when taking high-risk antibiotics. Zacharioudakis et al. noted a 21.8% CDI rate among hospitalized patients admitted with toxigenic *C. difficile* colonization independent of antibiotic risk, so this was our lower limit for an assumed conversion rate from colonization to infection. Based on current literature, we felt a 60% reduction in conversion to CDI after receiving the vancomycin treatment proposed in this study may be reasonably expected (34, 48, 82, 83, 85, 94). Group sample sizes of 44 in each arm achieve 80% power to detect a difference between the group proportions of 60%. Initially, the proportion in the treatment group was assumed to be 0.50 under the null hypothesis, and 0.20 under the alternative hypothesis. The proportion in the control group is assumed to be 0.50. The test statistic used is the two-sided Fisher's exact test, with the significance level set at 0.0500. However, since we are not sure what the frequency of CDI will be in either group, we completed various sample size calculations using PASS 13 Power Analysis and Sample Size Software, 2014 (NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass). Based on all of these variations (**Table 3**), our proposed sample size of 200 should be sufficient to detect a 60% difference between prophylaxis and placebo, allowing for at least 10% drop-outs if patients colonized with toxigenic *C. difficile* develop CDI when placed on high-risk antibiotics at least 30% of the time.

Table 3: Analysis of Two Independent Proportions Tests

Target Power	Actual Power	N (Arm 1)	N (Arm 2)	N (Total)	Assumed CDI in Placebo Group (P1)	Assumed CDI in Prophylaxis Group (P2)	Decrease in CDI (D1)	Target Alpha	Actual Alpha
0.80	0.80209	44	44	88	0.5000	0.2000	-0.3000	0.0500	0.0255
0.80	0.80171	64	64	128	0.5000	0.2500	-0.2500	0.0500	0.0298
0.80	0.80392	61	61	122	0.4000	0.1600	-0.2400	0.0500	0.0335
0.80	**0.80086**	**88**	**88**	**176**	**0.3000**	**0.1200**	**-0.1800**	**0.0500**	**0.0336**

Numeric Results of Tests Based on the Difference: P1 - P2 using Fisher's Exact test (H0: P1 - P2 = 0. H1: P1 - P2 = D1 ≠ 0), Selected sample size shown in bold.

3.2.2 Data Analysis

A. Data Collection and Data Management

A patient identification log will be kept to permit easy identification of each patient during and after the study. The patient identification log will be confidential and will be kept in a secure location in the study office. To ensure confidentiality, no copy will be made. The log will link the patient's name and medical record number to the unique study identification number. An electronic list of patients in the study, only available to study personnel, will be kept within the EMR of our healthcare system. Data will be collected using study-specific source documents in which the author of an entry is easily identifiable. The number of study personnel who will be responsible for data collection will be kept minimal to ensure the fidelity of the data. Study personnel will transcribe the data from source documents into a secure hospital-based computer system with password protection. All patient identifiers will be removed from the collected data to ensure HIPAA compliance and patient confidentiality. Patient data will be identified using initials and a patient study number. Any communication relating to the study, study documents, and electronic databases, will identify patients by initials and their assigned number only. In accordance with ICH/GCP guidelines, the study team will maintain all source documents that support the data collected from each patient as well as all study documents specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by appropriate regulatory requirements. All documents related to the study will be kept in a secure location in the study office, and will be preserved for a minimum of 11 years as required by internal policy in the study office or another approved location for long-term secure storage.

B. Data Monitoring, Quality Control, and Safety

This study protocol will employ rigorous methods to ensure data safety. Human subjects' safeguards will include, at a minimum, NIH Computer Security Awareness Training, completion of Human Subjects Protection Training, and signing a legally binding Assurance of Confidentiality. This study is felt to be minimal risk, however, Roger MacArthur, an attending infectious diseases physician from an outside group with significant research and data safety monitoring experience will serve as the Medical Monitor for the trial, and

will review Adverse Events and Significant Adverse Events for data safety monitoring for the duration of the proposed project. In the event that any study participant has a significant adverse reaction related to the study, the PI will, if needed, break blinding in consultation with the lead research pharmacist, Dr. John Koerber, and the medical monitor. Standard of care treatment for the participant will be followed.

C. Proposed Statistical Analysis

Descriptive statistics will be given for all data collected. Missing data will not be replaced by substitutions or interpolations. Categorical variables will be reported as counts and percent frequencies. Continuous variables will be examined for normality. Normally distributed variables will be provided as mean +/-SD (standard deviation) followed by the median. Non-normally distributed variables will be provided as medians, 25th, and 75th percentiles. We will compare the two randomized arms for demographics, ages, laboratory results, and other collected data, to determine any imbalances between the arms. Categorical variables will be examined using Pearson's chi-square tests where appropriate (expected frequency>5), otherwise Fisher's exact tests will be used. Normally distributed continuous variables will be examined with t-tests, while non-normally distributed continuous variables will be examined with non-parametric Wilcoxon rank tests. The 95% confidence limits and odds ratios (or relative risks) will be calculated wherever appropriate.

The total number of non-randomized patients who did not develop CDI will be reported as count and percent frequency. The *primary endpoint* of development of CDI will be examined between the two randomized arms using a Fisher's exact test, and will be reported along with the odds ratio and 95% confidence limit. The *secondary endpoints* of severity of CDI, rate of conversion from colonization as related to presence of *C. difficile* toxin IgG, and rate of colonization with VRE after prophylaxis with vancomycin will be examined with Pearson's chi-square tests where appropriate (expected frequency >5), otherwise Fisher's exact tests will be used. Various exploratory analyses, such as impact of age, impact of comorbidities, and mortality, may be completed to explore relationships with CDI and other data collected. These analyses will be completed using appropriate statistical methods by a Beaumont research biostatistician using R or The SAS® System for Windows Version 9.3 or higher (Cary, NC).

3.2.3 Randomization

Once patients have met all inclusion and exclusion criteria and have signed consent, they will be randomized in a 1:1 ratio of prophylactic vancomycin vs. placebo. This is a double-blind study, and the patients and their caregivers will not have knowledge of the arm to which the patient will be randomized. A randomization list will be prepared by a Beaumont research biostatistician in varying block design. This will insure that the number in both groups will be equal periodically. The list will be provided to the research pharmacist. He will provide the medications prepared by the Research Pharmacy for the patients in the sequential order according to the randomization list provided.

3.3 Potential Problems and Approaches to Minimize Their Impact

3.3.1 Low Recruitment

Our estimate for recruitment is based on prior experience in conducting multiple clinical trials and the size of the target population. Since vancomycin has been approved and marketed for years, the perception of risk associated with this clinical trial is reduced and one of the major roadblocks to recruitment has already been removed. However, given the size of the eligible population in our institution, if recruitment is below the needed 1.7 patients per day, additional efforts to approach larger numbers of patients will be undertaken.

3.3.2 Low Estimation of the Asymptomatic Toxigenic Carrier State

Our estimate of the percentage of patients who are asymptomatic carriers of toxigenic *C. difficile* is based on multiple prior studies in several different types of institutions (41, 52, 53, 55, 57, 95-98). The assumed 8% carrier rate is a conservative estimate. However, if the carrier rate is significantly lower than expected, this issue should become apparent early in the study, as screening for carriage is not blinded. To address this, we would plan to increase screening. However, prior to taking this step we will contact the program manager at AHRQ to discuss the issue and determine if increased screening can be accommodated within the budgetary considerations.

3.3.3 Low Estimation of Conversion of the Asymptomatic Carrier State to CDI

Our estimate of the percentage of asymptomatic carriers of *C. difficile* who will develop CDI when exposed to antibiotics is a conservative one based on multiple studies of development of CDI in colonized patients (48). Our study limits recruitment to patients started on high-risk antibiotics and the conversion rate

may actually be higher. It is reasonable to expect that the hypervirulent strain would be more likely to convert from asymptomatic carriage to CDI than previous strains. In our institution, the hypervirulent strain accounts for 60% of CDI (24), which we would expect to be reflected in the conversion rate.

3.3.4 Low Effectiveness of the Intervention

If the effectiveness of the intervention is significantly lower than expected, we will not be able to reach significance with the study as planned. However, given existing data (34, 82, 83, 85, 94), we have chosen a conservative level of effectiveness that should be reachable.

3.3.5 Difficulty in Establishing a Diagnosis of CDI

Since all patients in the randomized groups, by definition, had stool that tested positive for *C. difficile*, the use of the laboratory assay in defining CDI is limited. The diagnosis must therefore be made on a clinical basis. As such, any diagnosis of CDI will require a minimum of 2 days with ≥3 unformed stools per day, and toxin production will be determined for all patients who were determined to have CDI by EIA. Cases will be reviewed by Dr. Sims or one of the physician sub-investigators, all of whom have extensive experience in diagnosing and treating CDI. All investigators will be blinded to the treatment group.

3.3.6 Confounders from Outside the Study

Since patients in the trial will not generally be under the direct care of the study physicians, there is a possibility that care from outside physicians will interfere with the data integrity of the trial. First, should a patient be diagnosed with CDI by an outside physician and treated without clinical confirmation of the disease, the patient must be considered a CDI case though they may not truly have CDI. Second, any antibiotics started as an outpatient, during or after the receipt of the study medication, may cause a case of CDI. Despite these confounding issues, we must include these patients and count them as not having benefited from the therapeutic intervention. We will attempt to limit this problem by maintaining direct communication with treating physicians for inpatients and with primary care physicians for outpatients.

4. Future Directions:

If the **SToP CDI** study is successful, planned future studies will include the following: 1) a larger, multi-center trial will be conducted to confirm the results of this study; 2) patients known to be at risk for CDI, but with shorter healthcare exposures, such as those receiving antibiotic prophylaxis for surgical procedures, including bowel surgery, will be screened and treated prophylactically; 3) outpatient populations receiving high-risk antibiotics will be screened for *C. difficile* and treated prophylactically; and 4) alternative dosing or timing of prophylaxis using lower dose, lower frequency, or later initiation of prophylaxis for CDI will need to be studied in clinical trials to determine the most cost-effective strategies for CDI care.