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# Double blinded, randomized controlled Trial of Oral vancomycin versus placebo in hospitalized patients with diarrhea and stool toXin NEGative but nucleic acid amplification test positive for toxigenic Clostridium difficile (TOX NEG trial)

## Introduction

**Study Purpose:**
The purpose of this study is to determine the risks and benefits of antibiotic treatment for *Clostridium difficile* infection (CDI) among patients whose stool samples are nucleic acid amplification test (NAAT) positive and enzyme immunoassay (EIA) negative for *C. difficile*.

**Background:**
Clostridium difficile infection (CDI) is the most common cause of healthcare-associated diarrhea. There is no gold standard diagnostic test for (CDI). Commercially available assays detect *C. difficile* or its toxins in stool. Nucleic acid amplification tests (NAAT) are much more sensitive than toxin enzyme immunoassays (EIA). However, clinical correlation is needed to determine who has CDI. Most US clinical microbiology laboratories have adopted NAATs for *C. difficile* under the presumption the enhanced analytical sensitivity was beneficial. Although some patients with NAAT-positive/toxin-negative stool have CDI and a false-negative toxin EIA, subsequent studies indicate most patients with NAAT-positive / toxin-negative stool do not have CDI.[1-8] Rather, they are asymptomatic *C. difficile* carriers who have diarrhea for other reasons.[9,10] Most of these studies also have limitations and considerable controversy remains for whether NAATs or toxin EIAs should be used when CDI is suspected.[7,8,11,12]

We propose to conduct a double-blinded randomized controlled trial of CDI treatment for patients with NAAT-positive / toxin-negative stool. Such a trial is necessary to understand the risk-benefit of treating these patients for CDI. Patients with NAAT-positive / toxin-negative stool who consent to participate will be randomized to 10 days of oral vancomycin or placebo. Stool and environmental specimens will be obtained at regular time points and interrogated with culturomic and metagenomic methods. Patients will be followed until eight weeks after discontinuation of study drug. These data and specimens will be used to determine the impact of oral vancomycin versus placebo on the microbiome, *C. difficile* and MDRO colonization, environmental contamination, duration of diarrhea, CDI-related adverse events, and death.

**Specific aims and hypotheses:**

**Specific Aim 1:** Determine if there are differences in microbiome disruption and acquisition / persistence of *C. difficile* and other MDRO carriage in stool among patients with NAAT-positive / toxin-negative stool who are randomized to a 10-day course of oral vancomycin versus placebo.

**Hypotheses:** Study participants who receive oral vancomycin will have greater disruption of the taxonomic and functional metabolic profiles of the fecal microbiome, increases in antimicrobial resistance genes, acquire more MDRO, and will have greater persistence and abundance of MDRO in stool compared to participants who receive placebo. Participants who receive oral vancomycin will not have detectable *C. difficile* in stool after completion of study drug, but will be more likely to have *C. difficile* in stool at week 8 after completion of study drug compared to participants who receive placebo.

**Specific Aim 2:** Determine if there are differences in *C. difficile* and other MDRO environmental contamination between treatment groups.

**Hypothesis:** Study participants who receive oral vancomycin will have less environmental *C. difficile* contamination but more MDRO contamination compared to participants who receive placebo while receiving study drug. After

study drug is completed, those who receive oral vancomycin will have more environmental contamination due to both *C. difficile* and other MDROs.

**Specific Aim 3:** Determine if there are differences in CDI-related outcomes between groups.

**Hypothesis:** There will be no difference in time to resolution of diarrhea or CDI-related outcomes between treatment groups.

## Methods

**Study design:** Double blinded, randomized controlled non-inferiority trial of 10 days of oral vancomycin (125 mg 4 times per day) versus matching placebo for patients with NAAT-positive/toxin negative stool.

**Study population:** Adult patients whose stool was tested for *C. difficile* via EIA at Barnes-Jewish Hospital (BJH).

**Intervention:** The active study drug for this study will be oral vancomycin 125mg four times per day. The NAAT that will be used to screen toxin-negative stools is the Xpert C. difficile (Cepheid, Sunnyvale, CA), which is FDA-approved for testing of stool specimens collected from patient suspected of having CDI and the most commonly used NAAT in the US.[2]

**Sample size:** Due to the low frequency of CDI-related adverse events among NAAT-positive / toxin-negative patients, the best clinical outcome to assess response to therapy will be duration of diarrhea after randomization.[8] Existing data indicate the duration of diarrhea for NAAT-positive / toxin-negative patients is no different from NAAT-negative / toxin-negative patients, with 90% free from diarrhea at day 10.[8] Therefore the most appropriate design would be a non-inferiority trial, with the hypothesis that duration of diarrhea will be no different between oral vancomycin and placebo. To have 90% power and a one-sided alpha = 0.025, it would be necessary to enroll 190 study participants in each arm to have a non-inferiority margin of 10%; it would be necessary to enroll 757 participants in each arm to have a non-inferiority margin of 5% (PASS 14 Power Analysis and Sample Size Software (2015). NCSS, LLC. Kaysville, Utah, USA ncss.com/software/pass).

It is not feasible to enroll 380 study participants, collect and process specimens, and analyze the data in just one year, the time period available for this study. Therefore this study will evaluate the impact of treating NAAT-positive / toxin-negative patients on the fecal microbiome, MDRO colonization, and MDRO environmental contamination. The results of this trial will be used to justify sufficient time and funding to complete an adequately powered trial. For this trial, 50 evaluable subjects (per-protocol population – those who complete 10 days of study drug) are needed for analyses. 25 will be randomized to oral vancomycin and 25 will be randomized to placebo. In order to have 50 evaluable subjects, the targeted enrollment for this study is 80 patients.

**Inclusion criteria:** To be eligible, potential study participants must have stool submitted to the BJH microbiology laboratory for *C. difficile* testing that tests negative for *C. difficile* toxins (C. difficile Tox A/B II, Alere, Waltham, MA) as part of routine clinical care and positive by NAAT (Xpert C. difficile, Cepheid, Sunnyvale, CA), at least one diarrheal stool, and be ≥18 years of age.

**Exclusion criteria:** Patients who are not expected to survive until study follow-up is complete, have an allergy or intolerance to oral vancomycin, a history of CDI in the past 3 months, receipt of CDI antibiotic treatment (excluding empiric treatment given while pending EIA results), or does not provide consent will exclude a patient from participating in the trial.

Patients who are not able to provide consent will be eligible for the study if their legally authorized representative provides written informed consent and documentation of bowel movements is available.

**Recruitment:**

The charts of patients who have stool submitted to the BJH microbiology laboratory for *C. difficile* testing through routine patient care that are toxin-negative will be reviewed. Stools of patients who meet inclusion and exclusion criteria on chart review will be tested by NAAT (Xpert C. difficile, Cepheid, Sunnyvale, CA) by research staff. Patients whose stools are NAAT positive/toxin negative will be approached to participate in the study.

**Study Procedures:**
Patients will be approached in their hospital rooms by study personnel. Study personnel will explain the study and take time to answer any questions the patient may have. If the patient is interested in participating, written informed consent will be obtained.

Study personnel will perform an enrollment interview with the patient. Baseline data will be collected on demographics (e.g., age, sex, race), "typical" and current bowel movement consistency and frequency, healthcare exposures, dietary preferences, comorbidities, medication exposures, labs, and infection history. Chart review will be used to supplement patient interviews.

After the enrollment interview, patients will be randomly assigned in a 1:1 ratio to treatment groups using permutation blocks (n=4 per block), stratified by concomitant non-study drug antimicrobial use by computerized random number generator. The BJH investigational pharmacy will not be blinded to treatment assignment, but all other study personnel will be blinded.

After enrollment, patients will be contacted daily while on the study drug, and at weeks 4 and 8 after completion of the study drug, to determine bowel movement consistency and frequency, any new medication exposures, and assessed for adverse events, including diagnosis of CDI. If patients have been discharged from the hospital, they will be contacted via phone.

**Specimen collection:**
The qualifying NAAT-positive / toxin-negative stool specimen will be collected from the microbiology laboratory. In addition, stool will be collected at enrollment, days 5 and 10 while on study drug, and weeks 4 and 8 after completion of study drug (6 specimens / patient). If the patient is unable to have a bowel movement +/- 24 hours from the targeted collection time, and is admitted to the hospital, peri-rectal swabs will be collected.[13] If the patient has been discharged, he/she will be allowed +/- 72 hours to provide a stool sample. Swabs of the environment will be obtained at these time points as well. Three pre-moistened flocked swabs (BD, Franklin Lakes, New Jersey) will be held together, and surfaces will be vigorously sampled. Separate pairs of swabs will be used for each surface sampled. If the patient is hospitalized, the entire seat of the commode, a10cm x 10cm area of the bedside table, and a 10cm x 10cm section of the bedrail will be swabbed. If the participant is an outpatient, the entire seat of the commode, a 10cm x 10cm area of the kitchen counter, and a 10cm x 10cm area of the surface top where most meals are eaten will be swabbed. Disposable cut-outs for each site will be used to ensure consistent sampling. If the patient is hospitalized, study personnel will coordinate stool collection with the nursing staff. Once stool (or rectal swab) is obtained, the environmental surfaces will be swabbed. If the participant is not hospitalized, the participant will be provided with supplies and instructions to collect specimens, and then contact a courier to deliver the specimens to study personnel. We have considerable experience and standard operating procedures for this process, and have had near 100% compliance with study participant self-specimen collection, and no issues with loss of patient confidentiality with use of this courier service.

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