Exploratory Study of Impact of Oral Metronidazole, Vancomycin and Fidaxomicin on the Extent and Quantity of Host Carriage and Environmental Contamination with C. difficile

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Statistical Analysis Plan

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Study Design and Participants

We performed a prospective, unblinded randomized controlled trial of eligible and enrolled inpatients at Duke University Hospital. All adult inpatients diagnosed with CDI were eligible for inclusion; CDI was defined as a positive NAAT test in the setting of >3 loose stools in a 24-hour period. Eligible patients were excluded if prisoners, pregnant, required intravenous therapy for treatment of CDI, unable to consent, or received two or more doses of empiric therapy for CDI (with any agent). Eligible patients with no exclusion criteria were approached for enrollment.

Randomization

Enrolled patients were randomized to one of three study arms with 1:1:1 allocation: metronidazole 500mg PO q6, vancomycin 125mg PO q6, or fidaxomicin 200mg PO q12. Treatment was provided a minimum of 10 days following enrollment. Patients and providers were unblinded to study arm and treatment choice. The metronidazole treatment arm was considered to be the reference arm.

Outcomes

The primary outcome was the change in total environmental burden, measured as the total colony forming units (CFUs) of *C. difficile* isolated from each patient's hospital environment. Environmental sampling was conducted from 5 pre-specified locations (bedrails, overbed table, sink, toilet seat and

bathroom floor). 5 replicate Rodac plates (covering an area of 25 cm^2) were collected from each site. Secondary outcomes included total environmental burden (as measured by percent of hospital environmental samples positive according to each treatment group), *C. difficile* shedding over time (assessed as CFU/g of stool from serial patient samples), and molecular relatedness of isolates (assessed as the percent of environmental isolates sharing the same ribotype as the strain isolated from each patient's stool samples).

Study Procedures

Eligible patients were identified by microbiology-driven alerts or by orders for contact isolation for *C. difficile*. The study team was alerted of a potentially eligible patient. After confirmation of eligibility, the study coordinator approached the treating clinician and patient for potential enrollment and informed consent.

After obtaining informed consent, cultures were acquired from two sources: 1) environmental surfaces in the room and 2) stool samples from the patient. Cultures were obtained from these sources at predefined intervals starting the day of enrollment (day 0), days 3 and 7 following admission to the room, at the end of each subsequent week (days 14, 21, etc) and on the day of discharge from the hospital room (Figure 1). Environmental cultures were obtained from five high-touch environmental surfaces (bedside rail, bedside table, sink, toilet seat, and bathroom floor, toilet seat) and sampled in 5 replicates for each site.

Microbiological methods

The concentration of *C. difficile* in stool was obtained using ethanol shock followed by quantitative culture on CDSA plates to determine CFUs/g of stool on each sampling day. A minimum of five environmental sites were cultured in 5 replicates from each room using Rodac plates containing *Clostridium difficile* Selective Agar. All plates were anaerobically incubated at 37°C for 48 hours. For *C.*

difficile, the total number of CFUs of the targeted pathogen present on each plate was determined by identifying morphologies suggestive of the target organisms. These colonies were then sub-cultured and identity confirmed using a latex agglutination kit targeting *C. difficile*-specific cell wall antigens (Oxoid, UK).

We performed ribotying of *C. difficile* strains to assess whether environmental strains matched *C. difficile* shed by study subjects. Ribotyping was conducted on all initial patient stool specimens as well as environmental samples from each site. Ribotyping was performed according to the methods described previously by Bidet et al.[11] Cluster analysis to determine strain relatedness was conducted with Bionumerics imaging analysis software, using Dice coefficients and the unweighted paired group method with arithmetic mean (UPGMA). Band position tolerance was set at 1.25% and strains were considered identical if they exhibited a >99% match.

Statistical Methods

Standard descriptive statistics were used including medians and interquartile ranges (IQR) for non-normally distributed continuous variables. Comparisons of proportions were conducted with two-sample proportional z-tests.

To model the reduction in *C. difficile* shedding over time, stool or environmental *C. difficile* concentrations (CFU/g) were plotted for each subject over time. Inspection suggested an exponential decay curve. Concentrations (CFU/g) were then log transformed, providing linear decay curves for regression modeling. A linear mixed effects model (with subject identity as the random effects term) was employed to properly account for the correlation inherent in repeated sampling within subjects over time. All regression modeling was conducted in R (version 3.4.3) with the regression modeling package lme4.[12] Graphs were constructed using ggplot2.[13]