Microbial Diversity (Microbiome) Sub-Study

The microbiome is the collection of bacteria, fungi, viruses and eukaryotes that live on or in a host, as well as their genes and products. In a healthy state, the microbiome has numerous physiologically important interactions with the host. Disruption of its structure or function has been implicated in immune, inflammatory, metabolic and infectious diseases.
Antibiotic administration causes acute and precipitous loss of intestinal microbial diversity, with only incomplete recovery after their discontinuation. The effect of antibiotics on intestinal microbial composition and function varies with treatment duration and antibiotic type. Furthermore, the degree of healthcare-associated perturbation of the microbiome correlates with clinically relevant health outcomes including acquisition of antibiotic resistant organisms, nosocomial bloodstream infection and *Clostridium difficile* infection (CDI). This closely parallels the variable effects of antibiotic treatment duration and type from clinical studies.
Assays of microbial community diversity and structure represent a highly promising biomarker of antibiotic-associated risk of adverse outcomes. Here, we propose assessing intestinal microbial diversity in a subset of BALANCE trial patients in order to assess the effect of 7 vs 14 days of antibiotics on gut microbial composition.

Aims

Aim 1 - Comparison of the effect of 7 vs 14 days of antibiotics for bloodstream infection on microbial diversity. Rationale: Bacterial community diversity decreases after 1-3 days of antibiotic initiation. In one study of microbial community responses to a 5 day course of ciprofloxacin in healthy volunteers, bacterial diversity reached a nadir after 3-4 days of treatment and did not begin to recover until several days after cessation of antibiotics. In a longer study of a single patient, diversity reached a nadir at 11 days. The timing of these nadirs corresponds to the timing of peak elevations in the clinical risk of CDI detected in observational studies.
Hypotheses: i) Antibiotic administration will be associated with significant decreases in microbial diversity; ii) Longer duration of therapy (14 vs 7 days) will be associated with prolonged risk-associated decreases in bacterial community diversity, and; iii) Recovery of microbial diversity will be delayed in the 14 day treatment group.

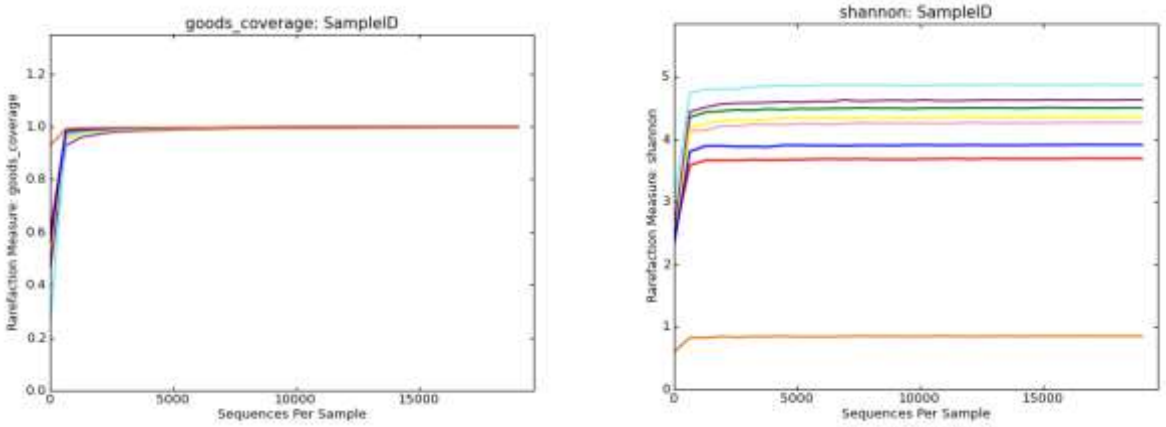
Aim 2 - Assess treatment specific effects on microbial diversity and community membership.
Rationale: Antibiotics with different mechanisms of action cause distinct patterns of change in bacterial communities and carry differing risks of CDI, with tetracyclines carrying the lowest risk, sulfa drugs, macrolides, penicillins, carrying intermediate risk, cephalosorins, monobactams, carbapenems and fluoroquinolones carrying further elevated risk, and clindamycin carrying the highest risk. Exposure to increasing numbers of antibiotics have additive effects on loss of bacterial diversity in human infections.
Hypotheses: i) Duration of treatment dependent loss of gut microbial diversity will be greatest for agents associated with high CDI risk, and; ii) Loss of bacterial diversity will be greater with increasing numbers of drugs and drug classes administered during a single course of treatment for BSI.

Methods

We will collect rectal swabs, after sampling just beyond the anal verge, from a subset of 190 patients (760 samples) from a subset of participating hospitals at the time of enrolment, day 7 and 14 of therapy, and at either discharge from hospital or day 28 post-enrolment, whichever is sooner. Dry swabs will be stored at -80^{o}C. Bacterial genomic DNA will be extracted using commercial column-based isolation kits prior to 16s rRNA gene amplification and sequencing on the Illumina platform at the Centre for Analysis of Genome Function and Evolution (CAGEF, University of Toronto) (see letter of support).
Sequence data will be quality-filtered and analyzed using the Qiime suite of microbiome analysis tools. Each sample will be analyzed for diversity (Shannon diversity index), richness (OTU number) and bacterial community membership. Sample communities will be compared using phylogenetic (weighted and unweighted UNIFRAC distance) and non-phylogenetic (Bray-Curtis dissimilarity) beta-diversity indices.

Sample Size Calculation for Microbiome Sub-Study
Normalized Shannon Diversity Index (SDI) (a measure of sample diversity) will be compared between 7- and 14-day treatment groups at each time point by student's t-test. Applying conservative estimates of a 10% percent reduction in SDI at the time of discharge, a 30% standard deviation, an alpha of 0.05 and a power of 0.90, we will require 190 patients at 4 time points (760 samples).

Preliminary data
Figure: Rarefaction curves for 16S rRNA sequencing of rectal swab samples. Rarefaction was performed in 500 sequence intervals for Good's coverage (A) and Shannon diversity index (B). Coloured lines represent individual samples.



A **B**

Figure: Bacterial diversity analysis of rectal swab samples. Shannon Diversity (A), observed taxonomic richness (B) and Simpson evenness (C) for taken at study enrolment (day 0) and subsequent sampling timepoints.