Developing Microbiome Rehabilitation Biomarkers for *Clostridium difficile* Infections: Evaluation of a Prototype Microbiome Health Index™

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Background
A healthy intestinal microbiome is important to human health, and microbiome disruption (dybiosis) is linked to many diseases, most notably recurrent *Clostridium difficile* infections (rCDI). This has spurred efforts to develop FDA-approved microbiota-based drugs to restore a healthier microbiome, including RBX2660, a standardized microbiota-based therapeutic with demonstrated clinical efficacy for preventing rCDI. However, quantitative biomarkers for microbiome restoration have not been established. Accordingly, we evaluated a prototype Microbiome Health Index (MHI) to describe microbiome restoration among patients treated with RBX2660.

Methods
MHI is a unidimensional parameter based on the association of Bacteroidia and Clostridia with colonization resistance and Gammaproteobacteria and Bacilli with dysbiosis. MHIs were calculated from shallow-shotgun sequencing data of fecal samples collected from patients enrolled and treated in an open-label Phase 2 evaluation of RBX2660 for preventing rCDI. MHIs were also calculated for the RBX2660 drug product and for Human Microbiome Project data (HMP). Receiver operator characteristic (ROC) analysis was used to define a MHI cut-point for distinguishing rCDI subjects prior to treatment (baseline) from the RBX2660 drug product. Post-treatment MHIs from patients were assessed longitudinally.

Results
The RBX2660 drug product and HMP data have similar MHIs, suggesting that RBX2660 is a suitable estimation of a “healthier” microbiota composition. Baseline MHIs were not significantly different between treatment responders or failures. ROC analysis indicated that pooled baseline samples could be distinguished from the RBX2660 profile (AUC=0.99, sensitivity=0.98, specificity=0.98, cutpoint=31, maximum odds ratio=46). At 10±4 days post-treatment, responder MHIs increased significantly compared to baseline ($p<.0001$) by an average of >5000-fold, and 49% of responders were above the MHI=31 cutpoint. Responder MHI values remained high 30 days after treatment. Statistical analyses for post-treatment failures were not possible due to low sample number.

Conclusion
MHI effectively distinguishes patients with dysbiosis from controls, and MHI increases can be measured post-treatment to demonstrate restoration toward a healthier microbiome composition. MHI statistical measures can readily derived and tested in prospective clinical trials. Future evaluation of this MHI will determine whether more specific taxonomic characterization and/or diversity measures increase the diagnostic value of MHI.

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Keywords: *Clostridium difficile* infection, RBX2660, microbiome rehabilitation

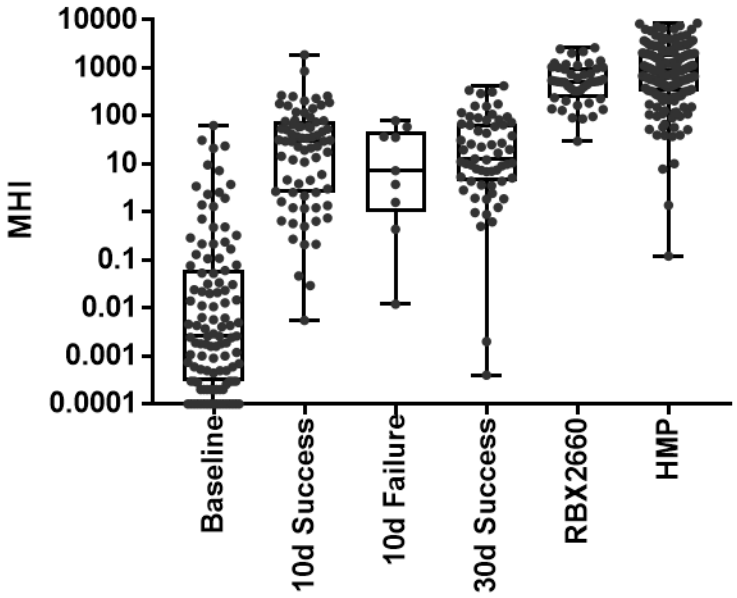

Figure 1. MHI for pre- and post-treatment, RBX2660, and HMP.