Utilization of host fecal micro-RNAs as a predictor of future *Clostridioides difficile* toxin production

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Introduction: Micro-RNAs play roles in gene regulation for both prokaryotic and eukaryotic genes. Recent studies have shown that human intestinal epithelial cells secrete exosomes that contain micro-RNAs. These exosomes are stable in the physiological environment and can directly affect bacterial growth. This lays the foundation for a deeper understanding into the relationship between a host and its microbiota. Utilization of fecal micro-RNAs can potentially serve as a tool to reason how and why gut dysbiosis can occur in humans how the host can control bacterial functions such as toxin production. CDI. a leading cause of morbidity and mortality, results from the production of exotoxins A and B. Factors that trigger the progression from asymptomatic *Clostridioides difficile* (CD) colonization to the symptomatic exotoxin production that hallmarks CDI has not been clearly elucidated. This project provides insight into this process by evaluating the regulatory role of micro-rna in the control of toxin production.

Hypothesis: We hypothesize that host derived miRNAs regulate CD toxin gene expression in CD bacteria

Methods: Human stool samples were collected at various time points during a chemotherapy regiment and stored in proper storage units. Stool samples were then tested for toxin B gene (tcdb) positivity using the Cephid Genxpert PCR test. Stool samples were then isolated following a modified MiRvana micro-RNA isolation procedure. Isolated micro-RNA samples were quantified using a micro-RNA assay with qubit measurement.

Results: Micro-Rna isolation data showed that there was no significant change in micro-RNA quantification levels pre and post chemotherapy initiation. Micro-RNA levels were able to be successfully isolated from stored human stool samples regardless of group. Micro-rna levels were consistent for the same sample across multiple isolations.

Conclusion: Isolation of miRNA from stool was feasible

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