Metagenomic Analysis of Human Fecal Microbiome Using Ion Torrent 16S rRNA Sequencing to Understand Microbial Diversity and Pathogen Association During Diarrheal Illness

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A total of 59 human stool samples associated with gastrointestinal illness were collected from 5 geographic sites (Oklahoma, Tennessee, Wisconsin, Iowa) and were shipped frozen to the MHD laboratory.

DNA was extracted on EasyMag (BioMérieux, NC), screened for GI pathogens using tXAG Gastrointestinal Pathogen Panel (GPP) (Luminex Corp. TX) and read on Bioplex 200 instrument (Bio-Rad, CA).

PCR primers 515/806 were used by Larkin Hospital to sequence 16S rRNA gene in a single-step 30 cycle reaction using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). Ion 318 chip (4-5.5 million reads) was used on Ion Torrent PGM (Life Technologies, CA) following the manufacturer’s guidelines. Operational Taxonomic Units (OTUs) were defined by clustering at 3% divergence (97% similarity) and finalised using BLASTn against a curated database.

RESULTS

CONCLUSIONS

- Metagenomic analysis of human fecal microbiome using Ion Torrent 16S rRNA sequencing has allowed a better understanding of the bacterial diversity and distribution of microbial pathogens in fecal samples collected from patients with diarrheal illness.
- Future studies aimed at understanding the link between specific bacterial population with diarrheal disease and population health risks are warranted.
- Gut microbiome analysis may open up opportunities to support antibiotic resistance analysis and fecal transplant screening efforts to improve patient care, treatment and case management practices in clinical and public health agencies.

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