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

Sanjib Bhattacharyya[1*], Marcos A. Sanchez-Gonzalez[2†], Caitlin Liddiard[1], Jose Navidad[1], Anami Patel[3], Mike McDermott[4], David Warshauer[5], Lucy Desjardin[6], Steve Gradus[1], Daria Salyakina[7] and Jonathan Coffman[2]

[1]City of Milwaukee Health Department Laboratory, WI; [2]College of Biomedical Sciences Larkin Health Sciences Institute, South Miami, FL; [3]LeBonheur hospital, TN; [4]Oklahoma State Department of Health, OK; [5]Wisconsin State Laboratory of Hygiene, WI and [6]State Hygienic Laboratory University of Iowa, IA; [7]Center of Computational Science, University of Miami, Miami, FL.

PREFACE

- Overall goal of the present study was to assess the distribution of 16S rRNA gene sequences to identify and characterize the gut microbiome in fecal samples collected from symptomatic and non-symptomatic subjects.
- Gut microbiota is presumed to play a key role in human health and disease through its impact on nutrition, pathogenesis and immunology[1].
- Human fecal pollution provides a reservoir for hundreds of waterborne disease agents and contributes worldwide to significant morbidity and mortality[2].

MATERIALS AND METHODS

- A total of 59 human stool samples associated with gastrointestinal illness were collected from 5 geographic sites (Oklahoma, Tennessee, Wisconsin-2 and Iowa) and were shipped frozen to the MHD laboratory.
- DNA was extracted on EasyMAG (BioMérieux, NC), screened for GI pathogens using xTAG Gastrointestinal Pathogen Panel (GPP)[3, 4] (Luminex Corp. TX) and read on Bioplex 200 instrument (Bio-Rad, CA).
- PCR primers 515/806[5] were used by Larkin Hospital to sequence V4 variable region of 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 finalized using BLASTn against a curated GreenGenes database[6].

Correspondence:
* sbhatt@milwaukee.gov
† masanchez@larkinhospital.com

RESULTS

Pathogens Detected by Luminex GPP

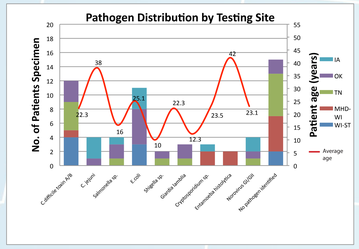

Figure 1. *Pathogens (10) identified in testing sites (total 42) using Luminex GPP. Age distribution (in years) for each pathogen highlighted by secondary axis. E. coli category included detection of ETEC, STEC, and O157:H7. 16S rRNA gene sequencing also confirmed all the bacterial pathogens detected using GPP assays.*

Overall Bacterial Diversity

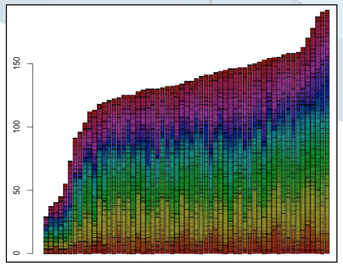

Figure 2: *Rarefaction curve analysis shows that 203 different species were identified in all the collected samples. Additional bacterial species associated with health and human diseases were also identified in the study samples.*

Bacterial Distribution Differences by Gender

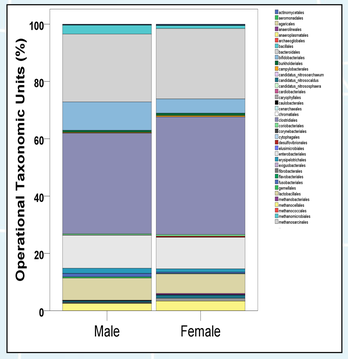

Figure 3. *Distribution of bacterial species (order) by gender. Student's t tests demonstrated significant ($p < 0.05$) gender differences such that Butyricimonas and Lactatifermentans were higher in females compared to males.*

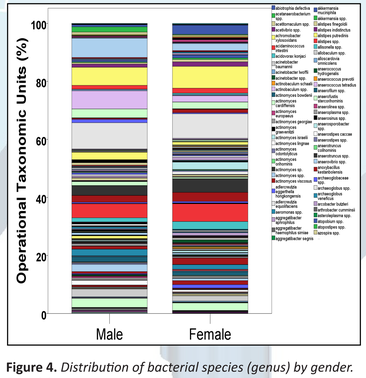

Figure 4. *Distribution of bacterial species (genus) by gender. Butyricimonas and Lactatifermentans bacterial species have been found to be associated with anti-inflammation properties and dairy consumption, respectively.*

Bacterial Distribution Differences by Site

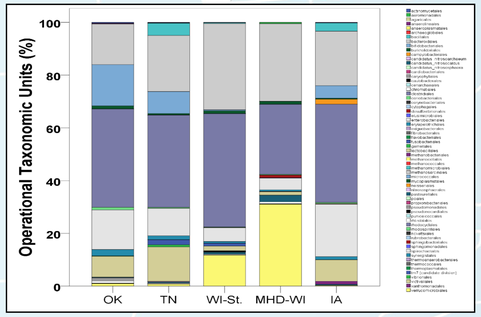

Figure 5. *Distribution of bacterial species (order) by site. The ANOVA analysis demonstrated a significant ($p < 0.01$) interaction. Follow up tests revealed that the Verrucimicrobiales species was higher ($P < 0.05$) in WI state samples and those from Milwaukee (SE WI) (~25%) specimens compared to the other sites.*

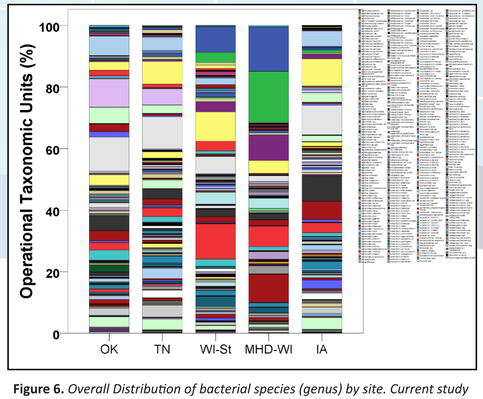

Figure 6. *Overall Distribution of bacterial species (genus) by site. Current study did not identify any significant gender vs. site interactions.*

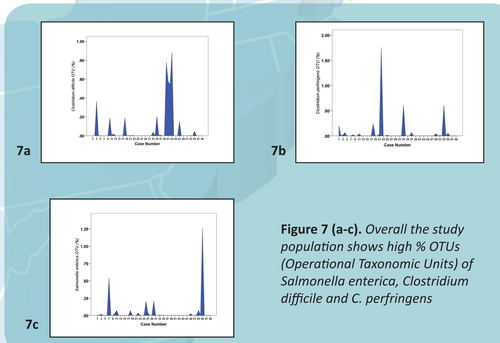

Figure 7 (a-c). *Overall the study population shows high % OTUs (Operational Taxonomic Units) of Salmonella enterica, Clostridium difficile and C. perfringens*

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 populations 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.

ACKNOWLEDGEMENTS

We acknowledge the staff at the City of Milwaukee Health Department, LeBonheur hospital, TN, Oklahoma State Department Health, WI State Laboratory of Hygiene, and State Hygienic Laboratory University of Iowa for collection of samples, laboratory analysis and outbreak investigations. We appreciate editorial assistance from Kwadwo Owusu-Ofori and Julie Becker at MHDL.

REFERENCES

[1]Young VB. 2012. Curr. Opin. Gastroenterol 28: 63–69.
[2]Fewtrell L, et. al. 2005. Lancet Infect Dis. 5: 42–52.
[3]Navidad J, et. al. 2013. J. Clin. Microbiol. 51: 3018–3024.
[4]Patel A, et. al. 2014. J. Clin. Microbiol. 52: 3068-3071.
[5]Caporaso JG et. al. 2011. Proc Natl Acad Sci U S A;108 Suppl 1:4516-22.
[6]DeSantis TZ et. al. 2006. Appl Environ Microbiol;72(7):5069-72

Presented at the 2015 APHL Annual Meeting, Indianapolis, IN