

.Project number: 6835
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GallBiome: Characterization of the Gallbladder Microbiome



Key external stakeholders:
health care professionals, patients

Practical implications for stakeholders:
Characterization of the core gallbladder microbiome has important biological and medical implications with potential to lower the risk and incidence of cholelithiasis.

Main results:

The human gastrointestinal tract harbors a complex community of microorganisms that confer metabolic, immunological and neurological benefits to the host. Scientists have begun to uncover the importance of these bacterial inhabitants and expand investigations to consider how site-specific microbiomes affect host physiology. While more than one million cholecystectomies (gallbladder removal surgeries) are performed throughout Europe each year, the bacterial communities associated with the human gallbladder and its disease states remain unknown. Results of the GallBiome project showed that metabolites extracted from the gallbladder mucosa, bile, and circulating blood plasma clustered according to disease pathology. Specifically, fatty acids were the primary driver of statistical differences, with increases in tetradecanoic acid, hexadecanoic acid, and octadecanoic acid driving the most changes.

Opportunity / Benefit:

The GallBiome project aimed to establish relationships between gallbladder microbiota, gut microbiota, and human health with a view to informing future development of diagnostics and therapeutics.

Collaborating Institutions:

Teagasc, UCC/APC Microbiome Ireland, Mercy Hospital, Cork University Hospital

Teagasc project team: Prof. Catherine Stanton
Dr. Crystal Johnson (Fellowship recipient)

External collaborators: Prof. Paul Ross (UCC/APC)
Mr. Cristoir O'Suilleabhain (MUH Upper GI and Hepatobiliary surgeon)
Mr. Bill Bennett (CUH Histopathologist)
Prof Noel Caplice (Cardiologist, Centre for Research in Vascular Biology)

1. Project background:

Gallbladder disease accounts for a substantial number of surgeries worldwide, ranking number one for the most frequently performed major surgery. In the U.S. direct and indirect costs of gallbladder disease represented a consumption of \$6.2 billion in 2012, increasing by more than 20% over the last three decades. While statistics for Europe collectively are more difficult to find, in 2013 England reported >12,000 cases of cholelithiasis, making it one of the primary reasons for hospital visits relating to gastrointestinal issues. As the number of people requiring gallbladder excision steadily increases, considerable burden is placed on the healthcare system and taxpayer funds. However, the degree of research in this topic does not reflect demand, and much remains to be discovered about the epidemiology of gallbladder disease. As studies continue to connect bacterial activities to host health, it is expected that a better understanding of the gallbladder microbiota will help shed light on cholelithiasis. Therefore, investigations that characterize this indigenous community and its functions in humans are warranted.

2. Questions addressed by the project:

This project sought to define the core gallbladder microbiome (adherent and non-adherent). It was hypothesized that measureable differences will be seen among the dominant phyla in healthy compared to diseased gallbladders and that removal of the gallbladder will lead to gut dysbiosis. To investigate these questions the following objectives were highlighted: 1) mapping community changes linked to gallbladder disease; 2) quantifying changes in bacterial communities from gallbladder mucosa, bile and stool as a function of time and patient status, 3) evaluation of gallbladder removal on the gut population (3) correlations among population profiles with metabolite to glean microbial functions, and 4) the isolation and characterization of previously uncultivated anaerobic bacteria.

3. The experimental studies:

Ethical approval was granted for project protocols, consent forms, clinical questionnaires, recruitment advertisements, information packets and clinical SOPs. Meetings and training sessions were held with clinicians to establish working protocols and hospital staff were trained to facilitate specimen sampling and handling. However, recruitment of participants proved difficult and those who consented often failed to bring stool samples to their surgery, were deemed inappropriate specimens in the theatre (due to visible polyps or liver cirrhosis which might confound results), or nursing staff incorrectly placed tissue samples into formalin. Therefore it was decided to also investigate the core gallbladder microbiome in pigs, as these variables could more be more tightly controlled in this animal model, which is similar in intestinal architecture and microbiota composition and function to humans. Twenty pigs were equally divided into two diet groups, fed either a normal chow (NC) diet or a "Western Diet" (WD) supplemented with 4% salt, 4% cholesterol, 25% crude fat, 0.5% cholate and 26% sugar for a period of 12-weeks. Faecal samples were collected on a weekly basis and the effects of diet on metabolic parameters and the intestinal microbiome using 16S Metagenomic sequencing were investigated. At 12 weeks, the pigs were culled and evaluations of the internal organs took place, including gallbladder, bile and blood plasma.

4. Main results:

This study extends a prior porcine model of cardiometabolic syndrome to include systemic inflammation, fatty liver, and insulin sensitivity. Gallbladder and bile metabolomics data revealed vast differences in the circulating amino acids, lipids, and fatty acids between the two groups. Gut microbiome changes during evolution of porcine cardiometabolic disease recapitulate those in human subjects with alterations in gut taxa associated with proinflammatory bacteria, bile acid, and fatty acid pathways. Methodologies were developed for the collection and curation of microbes from diseased/healthy gallbladders, bile and stool specimens.

5. Opportunity/Benefit:

The gall bladder microbiome is an important source of health-related biomarkers. A correlation between disease pathology and circulating fatty acids was shown, which has implications for healthcare diagnostics and management of care. While the impact of gallbladder removal on the gut microbiome has yet to be revealed, this work is relevant to the clinicians managing cholecystectomies as well as patients who can make alternative dietary choices. Future studies will seek out the mechanisms at play, in an effort to distinguish cause-or-effect relationships between gallbladder fatty acids and gallbladder disease.

6. Dissemination:

Davidson, G. L., Cooke, A. C., Johnson, C. N., & Quinn, J. L. (2018). The gut microbiome as a driver of individual variation in cognition and functional behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1756), 20170286.

Johnson, CNJ and Valoo, ACM (2019). Peptostreptococcus, Finegoldia, Anaerococcus, Peptoniphilus, Veillonella, and other anaerobic cocci. In *Manual of Clinical Microbiology, 12th Edition* (pp. 803-816). American Society of Microbiology.

Popular publications:

- Speaker at the *APC New Year Symposium* (Jan 12th, 2017 at the Clayton Hotel, Cork)
Talk title: The Uncultivated Majority
- Speaker at the APC Forum – “*APC Celebrates Marie S. Curie Fellows*” (Jul 18 2017)
Talk title: Characterisation of the Human Gallbladder Microbiome
- Speaker at the *Proposal Writing Workshop for Marie Skłodowska-Curie Individual Fellowships* (July 31st, 2017 at Teagasc Head Office, Oak Park)
Talk title: GallBiome: Tips and Tricks from a Funded Fellow
- Speaker at the *Post-Doctoral Development Programme workshop “Successful H2020 Applications, an Evaluator’s Perspective”* (July 12th, 2017)
Talk title: My Marie Curie Journey
- Speaker at the *Teagasc Post-Doctoral Grant Writing Course* (June 8th, 2018)
Talk title: Acquiring H2020 funding, how to do it and what it means for your life
- Research Highlight in the Winter Edition of APC Microbiome Institute’s publication, *Gut Reaction Issue 13* (December 6th, 2016. http://apc.ucc.ie/?wysija-page=1&controller=email&action=view&email_id=11&wysijap=subscriptions).
- newspaper article featured in *The Irish Times* titled Bacteria and Bugs in Guts and Gallbladders appear in the ‘Researchers’ Lives’ column (Oct 27, 2016)
<http://www.irishtimes.com/news/science/bacteria-and-bugs-in-guts-and-gallbladders-1.2833102>).
- Dr. Johnson was profiled in a special edition titled “Teagasc Women in STEM,” in the Teagasc publication, *TResearch* (Nov. 10th, 2017).

7. Compiled by: Catherine Stanton