An overview of the similarities and differences between 11 body sites in cirrhotic patients using 16S Metabarcoding

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INTRODUCTION

- Thanks to its large scope, the Mucosa Predict cohort makes it possible to study the different microbiomes in the bodies of cirrhotic patients in great detail.
- 16S Metabarcoding was performed on over 1,400 samples taken across 11 body sites from the 93 cirrhotic patients of the Mucosa Predict cohort over multiple clinical visits. An additional 200+ technical controls were included in the study to ensure the quality of the results.
- This poster aims to give an overview of observable similarities and differences between tissues. Correlation with clinical data will be further studied in the 16S paper.

MATERIAL AND METHODS

- Sequencing was performed by LUMC (saliva), EMBL (feces) and Vaiomer (gastrointestinal biopsies and buffy coat) using Illumina MiSeq technology (2x300 and 2x250bp).
- 16S sequences of all samples and controls were clustered into Operational Taxonomic Units (OTUs) using the Swarm algorithm (adaptative clustering threshold) and analysed using Vaiomer's metagenomic pipeline
- Here are the 11 studied sample types and the colors used in subsequent figures:

Saliva	Fundus Antrum Bulbus Duodenum	Terminal Illeum Right-Colon Sigma Rectum	Feces	Buffy Coat
	(Upper Gastro Intestinal tract)	(Lower Gastro Intestinal tract)		

RESULTS

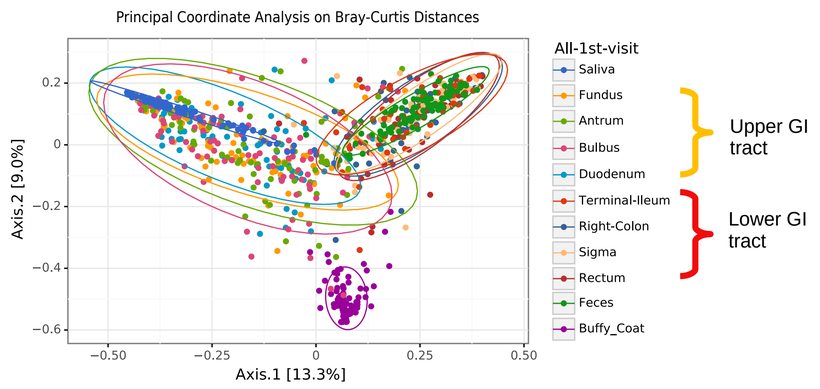

Figure 1: Beta Diversities show clear separation between tissue types. The four upper GI tract biopsies share strong similarities. They seem to generally be similar to saliva, but are also distributed between the other tissue types. The four lower GI tract biopsies also share strong similarities, and distribute close to the feces samples. Buffy coat samples show a clear separation from other body sites.

Principal coordinate analysis on beta diversities using the Bray-Curtis distances of samples taken during the first available visit for each patient.

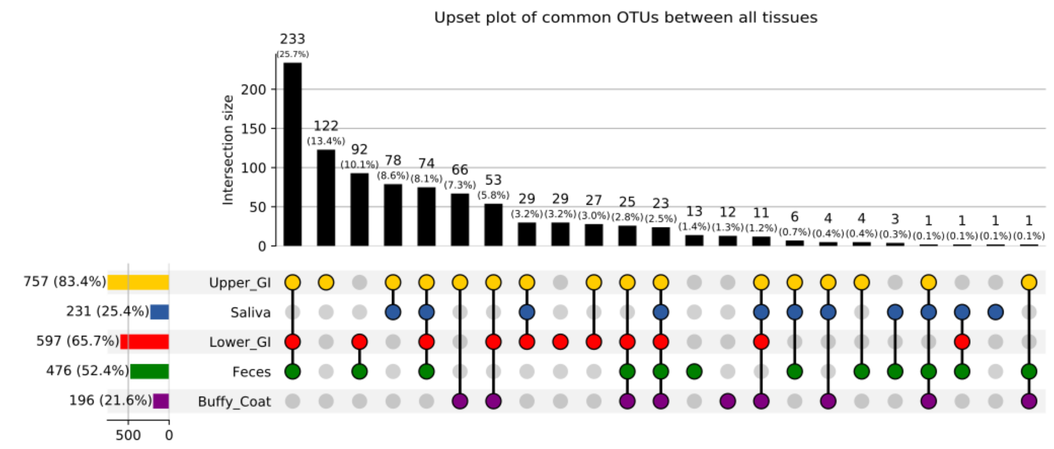

Figure 2: Different tissue sites share many OTUs. Upper GI tract and lower GI tract samples contain the highest total number of OTUs. Upper GI tract samples also contain the most unique OTUs. Feces share most OTUs with both GI tracts. Buffy coat and saliva contain the fewest OTUs, which are mostly shared with the Upper GI tract.

Upset plot of samples taken during the first available visit for each patient displaying shared OTUs between sample types. The ANCOM-II structural zero detection method was used to remove OTUs present in too few samples within each sample type.

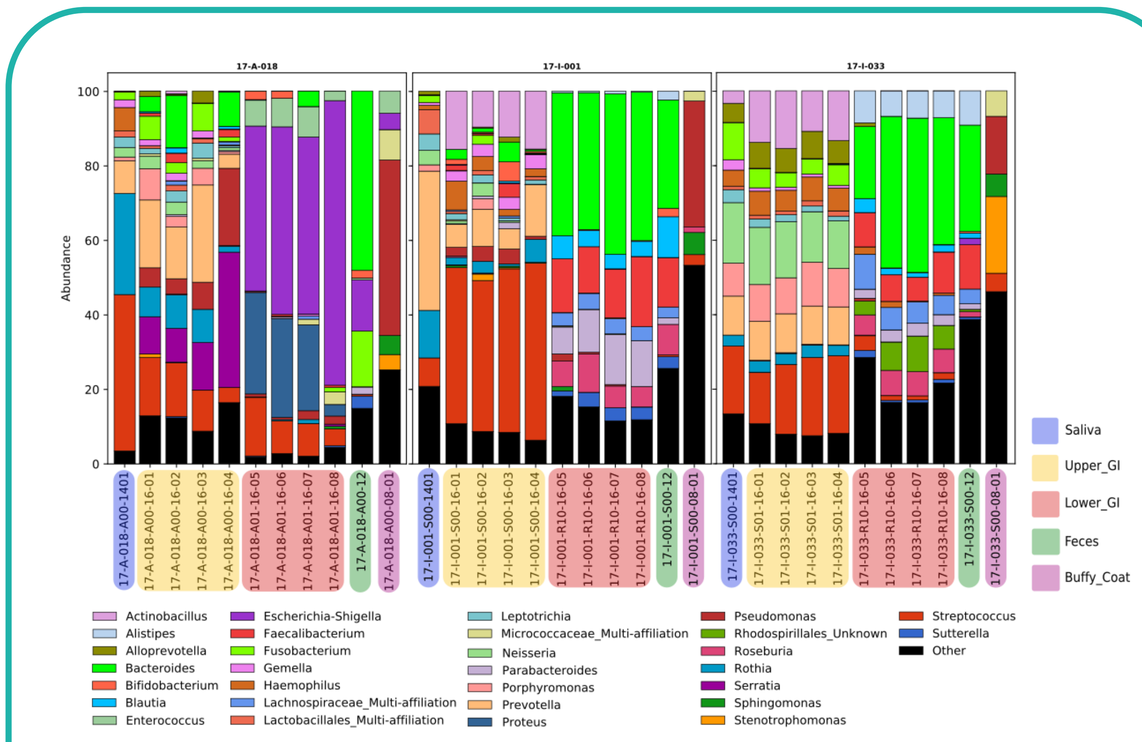

Figure 3: Taxonomic profiles at genus level show high variability between patients and between tissue types. These three examples illustrate similarities between upper GI samples and similarities between lower GI samples within each patient. There are also clear similarities between saliva and upper GI samples and similarities between feces and lower GI samples. Buffy coats have specific profiles different from other sample types and are in accordance with profiles found in the literature.

Taxonomic compositions showing the top 30 most abundant genera for three selected patients (17-A-018, 17-I-001 and 17-I-033). Only samples taken during the earliest available clinical visit of each patient are displayed.

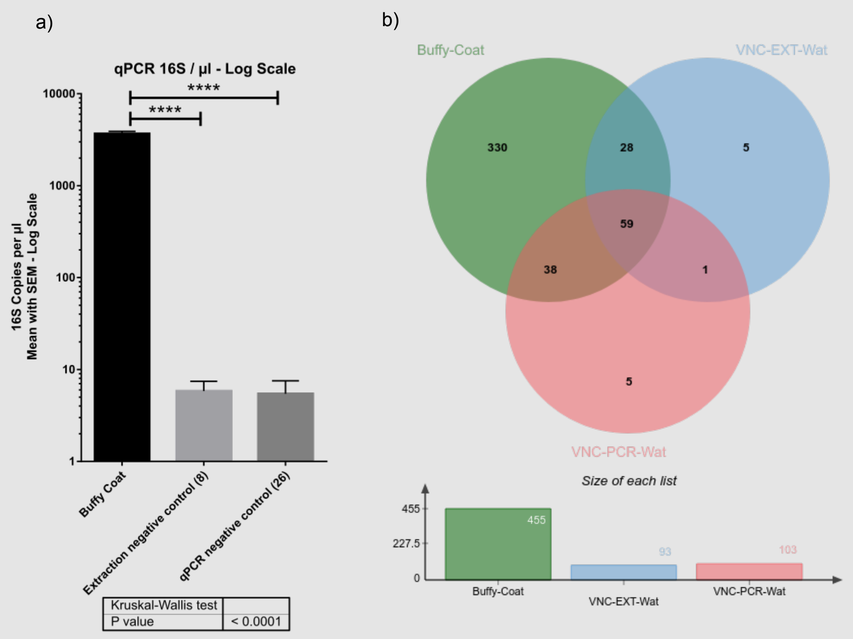

Figure 4: Buffy coat samples contain significantly more bacterial DNA than negative controls (3-4 log difference). Buffy coat also has higher taxonomic richness than controls: around 70% of OTUs present in buffy coat are not present in negative controls.

*Comparison between all 316 Buffy-Coat samples and 34 negative controls. Extraction Negative Control: molecular grade water extracted, amplified and sequenced at the same time as the buffy coat samples. qPCR/PCR Negative Control: molecular grade water amplified at the same time as the buffy coat samples without prior DNA extraction. **** : $p < 0.0001$ with Mann-Whitney test*
The ANCOM-II structural zero detection method was used to remove OTUs present in too few samples within each sample type..

CONCLUSIONS

- To our knowledge this study is among the first to analyse the microbiomes of such diverse sample types in a single patient.
- There are strong differences between patients and between certain tissue types in terms of diversity and taxonomic profiles.
- Similarities between tissue types seem to follow anatomical proximity. It would be interesting to see if the microbiomes in the small intestine form a gradient between the upper GI tract and colon.
- In terms of OTUs, the upper gastro intestinal biopsies display the highest taxonomic richness and share a significant proportion of OTUs with all other tissues.

Overall this study illustrates the richness and complexities of the different microbiomes studied in this cohort. The variability between patients, sample types and clinical visits could be a rich source of biomarkers, but it is also a major technical challenge for the correlation with clinical outcomes.

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