Identification of a Core Bacteriome that is associated with HIV Reservoir Size and Activity Across the Human Gut Segments

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Background: Each gut segment has a different local immune and bacteriome environment. This regional variability may limit the effectiveness of interventions targeting the bacteriome on the largest HIV reservoir in the human body.

Methods: Tissue samples across 5 gut segments (duodenum, jejunum, ileum, colon, rectum) were collected during rapid autopsies (≤6 h from death) from people with HIV enrolled in the Last Gift cohort. For each segment, we determined the bacteriome composition by 16SrRNA sequencing and measured cell-associated HIV DNA and RNA by ddPCR. Bacterial α and β diversity were analyzed (Phyloseq R). Dimension reduction of taxa was performed by filtering by thresholds of relative abundance (RA), prevalence, and number of reads. Mixed-effect models assessing the associations between the RA of taxa and both HIV DNA and RNA were adjusted for gut location and other relevant factors.

Results: 24 participants were included (87.5% male, 63±13 years, median CD4 T cells 194/μL [97-347], 87.5% virally suppressed). Main cause of death was cancer (66.7%; 4 of the gut). We observed a large heterogeneity in the bacteriome composition across each taxonomic level and among even anatomically adjacent segments. Significant differences in α and β diversity were observed between upper and lower gut and even among adjacent segments. Similarly, HIV DNA levels (but not HIV RNA) differed between upper vs lower segments (101 vs 40 cp/10^6 cells, p=0.003). In adjusted models, 17 taxa were associated with HIV reservoir measures independently from gut location (Fig.1). Of note, in segment specific models, none of these taxa was consistently associated with reservoir measures across all the segments: e.g., *Clostridium* genus was associated with higher HIV RNA in duodenum, ileum, and rectum only (p<0.02 for all). While others showed opposite associations across tissues: e.g., *Lachnospira* genus was associated with lower HIV DNA in colon but higher in ileum (p<0.01 for both), *Barnesiella* and *Ruminococcus* genus were associated with higher HIV RNA in duodenum but lower in rectum (p<0.03 for all).

Conclusions: Our study illustrates the potential impact of local bacteriome on HIV persistence in the gut. The segment-specific differences in bacteriome composition and in their relationships with local HIV reservoir may hinder microbiome-based interventions on the gut reservoir. Further studies are needed to identify target bacteria that can be effectively addressed in all gut segments.

Figure 1. Heatmap of the significant associations between bacterial taxa and gut tissue levels of HIV DNA and HIV RNA

Bacterial Taxa (Relative Abundance) significantly associated with gut HIV DNA levels				
Phylum	**Class**	**Order**	**Family**	**Genus**
Fusobacteriota	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacteriaceae f
Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	-
Bacillota	Clostridia	Clostridiales	Lachnospiraceae	Lachnospira
				Ruminococcus
			Ruminococcaceae	Ruminococcaceae f
			Clostridiaceae	Dorea
	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus
Pseudomonadota	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	-
	Alphaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas
	Gammaproteobacteria	Alteromonadales	Alteromonadales o	Alteromonadales o
Bacteroidota	Flavobacteria	Flavobacteriales	-	-
	Bacteroidia	Bacteroidales	Barnesiellaceae	Barnesiellaceae f

Legend scale (gut HIV DNA): +200, +100, +50, +20, +5, aβ, -5, -20, -50, -100, -200

Bacterial Taxa (Relative Abundance) significantly associated with gut HIV RNA levels				
Phylum	**Class**	**Order**	**Family**	**Genus**
Bacillota	Clostridia	Eubacteriales	Clostridiaceae	Clostridium
			Lachnospiraceae	Lachnospira
	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae f
				Coprobacillus
	Negativicutes	Acidaminococcales	Acidaminococcaceae	Acidaminococcus
Pseudomonadota	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	Proteus

Legend scale (gut HIV RNA): +50, +25, +20, +15, +10, +5, aβ, -5, -10, -15, -20, -25, -50

Significant (p value<0.05) associations between relative abundance of bacterial taxa (fixed effect) and reservoir measures (gut HIV DNA above; gut HIV RNA below) are shown. Each model included participant random effect and was adjusted by age, sex, current and nadir CD4+ T cell count, duration of HIV infection, last plasma HIV RNA, and gut segment. Legend: f/o, taxa attributed at the family/order level for uncertainty of attribution at the lower taxonomic level; aβ, adjusted beta coefficient.