Anal Microbiome Dynamics in MSM of the DEPIST-H Cohort: HSV-Linked Dysbiosis and HR-HPV Persistence

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Victor EUZEN[1,2], Arnold Junior SADIO[3], Maud SALMONA[1,2], Thibault SAINT-JOANNIS[4,5], Romane GUILBAUD[4,5], Fifonsi GBEASOR-KOMLANVI[6], Séverine MERCIER DELARUE[2], Sonia BURREL[7,8], David BOUTOLLEAU[9,10], Anoumou Claver DAGNRA[11], Diane DESCAMPS[4,5], Jade GHOSN[4,12], Didier Koumavi EKOUEVI[3,6], **Charlotte CHARPENTIER[4,5]**, Valentine Marie FERRE[4,5]

1.Université Paris Cité, Équipe Biologie et Pathogenèse des Virus, INSERM U944, Institut de Recherche Saint-Louis, 75010, Paris, France; 2. Service de Virologie, Hôpital Saint-Louis, APHP, France; 3. ISPED, Université de Bordeaux & Centre INSERM U1219 - Bordeaux Population Health, Bordeaux, France; 4. Université Paris Cite, INSERM UMR1137, IAME, 75018, Paris, France; 5. Service de Virologie, Hôpital Bichat-Claude Bernard, AP-HP, France; 6. Département de Santé Publique, Université de Lomé, Faculté des Sciences de la Santé, Lomé, Togo; 7. CNRS UMR 5234, Fundamental Microbiology and Pathogenicity, Université de Bordeaux, Bordeaux, France; 8. Service de Virologie, CHU de Bordeaux, Bordeaux, France; 9. Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et de Santé Publique, Paris, France; 10. Service de Virologie, Hôpital de la Pitié-Salpêtrière, AP-HP, Paris, France; 11. Laboratoire de Biologie Moléculaire et d'Immunologie (Biolim), Université de Lomé, Faculté des Sciences de la Santé, Lomé, Togo; 12. Service de Maladies Infectieuses et Tropicales, Hôpital Bichat-Claude Bernard, AP-HP, France.

BACKGROUND

The microbiota represents a dynamic balance between bacteria associated with **host homeostasis** and bacteria linked to **pro-inflammatory states**. The role of herpes simplex virus (HSV) in shaping the anal microbiome and promoting high-risk human papillomavirus (HR-HPV) persistence remains poorly understood. In this context, the aim of this study is to explore the dynamics of the anal microbiome in a population of Men having Sex with Men (MSM) in the context of HSV infection.

METHODS

Our study is based on the DEPIST-H ANRS 12400 cohort, which was established in 2021. The cohort included 200 MSM in Togo, 50% of whom with HIV (WIHV) at inclusion. Participants were followed for 2 years with anal samples collected: at inclusion (M0), 12 months (M12), and M24. Anal samples were screened for HSV by PCR at each timepoint for all participants. HSV serology was done at baseline (M0). A total of 32 participants presented at least 1 timepoint positive for HSV by PCR.

Then, we explored the anal microbiome by DNA shotgun metagenomic sequencing on 79 anal samples from 30 participants with ≥1 HSV-positive swab (exclusion of 9 samples with low sequencing read counts and 2 samples from 2 participants without longitudinal follow-up). Participants were stratified in primary (PI) or non-primary (NPI) HSV infection groups (seronegative or seropositive for HSV at M0 respectively) (Figure 1). HR-HPV persistence (PCR detection) was defined as 2 consecutive HPV-positive samples for the same HPV type.



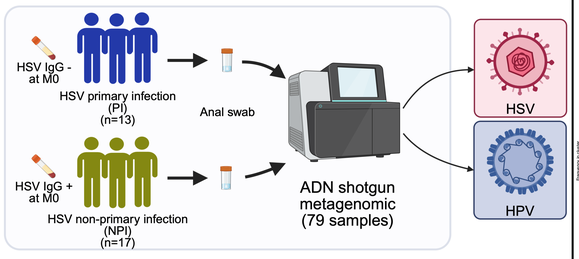


Figure 1 – Workflow of the study

Temporal dynamics of the anal microbiome differs between HSV primary and non primary infections groups, while HR-HPV persistence aligns with pro-inflammatory dysbiosis signatures

RESULTS

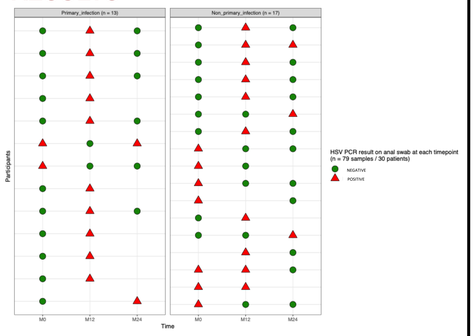


Figure 2– Longitudinal analysis of anal swabs using HSV PCR at the different timepoint (M0, M12, M24)

Changes in microbial composition (β diversity) are observed over time in the HSV PI group, whereas the microbiota remains stable in the HSV NPI group

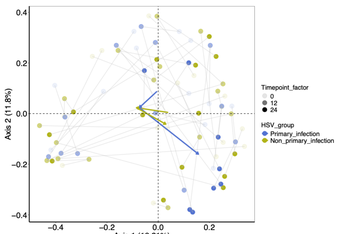


Figure 3 – Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity illustrating the temporal evolution of microbiota composition across the 3 timepoints within HSV PI and NPI groups

Unsupervised clustering identified 5 distinct microbial clusters (1 to 5) based on overall community microbiota composition

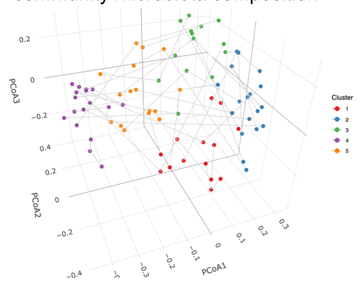


Figure 4 – 3D dot plot showing the coordinates of each sample along the first axes from a PCoA based on Bray-Curtis dissimilarity
Grey lines connect samples from the same participant

Cluster 4 is enriched in HSV-positive samples

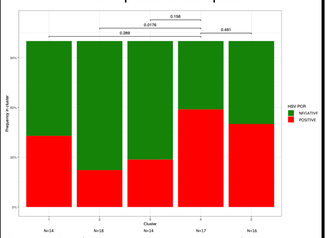


Figure 5 – Bar plot representing HSV positive and negative samples proportions in clusters (Fisher, p = 0.132)
N = number of samples

Depletion of "protective" genera, *Prevotella*, and *Campylobacter*

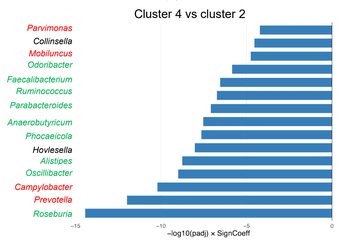


Figure 6 – Bar plot showing the top 15 genera with significantly different relative abundances between cluster 4 and cluster 2

Both groups show a dysbiotic dominance, with increasing ***Finegoldia*** and ***Streptococcus***, steady state for ***Faecalibacterium*** and ***Agathobacter***, and group-specific trends for ***Gardnerella*** and ***Bacteroides***

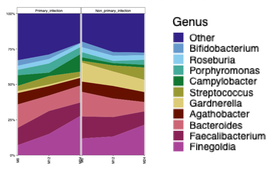
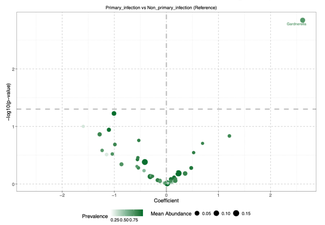


Figure 9 – Stacked plot showing relative abundance of the 10 most abundant genera across 3 timepoints in HSV PI and NPI

Gardnerella shows a significantly different temporal dynamics between the HSV PI and HSV NPI groups



HR-HPV persistence was not associated with global diversity shifts but showed temporal enrichment of inflammation-linked genera (***Parvimonas***, ***Fusobacterium***, ***Anaerococcus***).

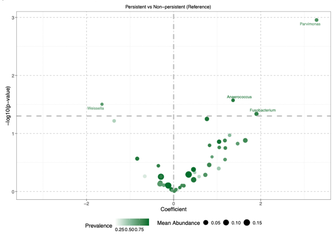


Figure 11 – Volcano plots displaying the results of longitudinal differential abundance testing for genera, comparing temporal trajectories between HSV PI and NPI (upper), HPV-persistant and non-persistant (lower)

Cluster 1 is enriched in HSV NPI samples

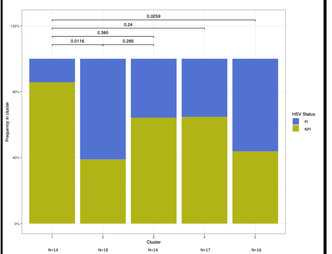


Figure 7 – Bar plot representing proportion of PI and NPI in each cluster

Cluster 1 is enriched in pro-inflammatory taxa and depleted in several genera associated with homeostasis

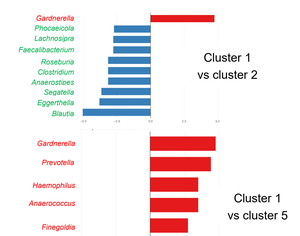


Figure 8 – Bar plots exhibiting genera with significant relative abundance between cluster 1 and cluster 2 (upper) or cluster 1 and cluster 5 (lower)

18 out of 30 participants presented persistent anal HR-HPV infection

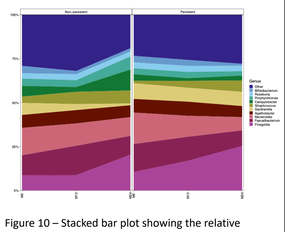


Figure 10 – Stacked bar plot showing the relative abundance of the 10 most abundant genera across three time points in HPV-persistent and non-persistent groups

PATHOPHYSIOLOGICAL HYPOTHESIS

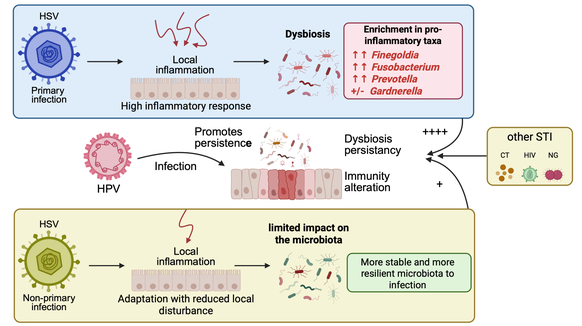


Figure 11 – Schematic representation of the pathophysiological hypothesis

CONCLUSIONS

Temporal dynamics of the anal microbiome appeared to differ between HSV PI and NPI groups while HR-HPV persistence aligns with **pro-inflammatory dysbiosis** signatures.

In addition to HSV impact on microbiome, these findings support virus-anal microbiome interactions as contributors to HR-HPV persistence and highlight the need for integrative studies of host–microbiome–virus dynamics in this context.

CONTACT ADRESS

charlotte.charpentier@aphp.fr

victor.euzen@aphp.fr

PLAIN LANGUAGE SUMMARY

Our results suggest that primary anal HSV infection may be associated with dysregulations in the anal microbiome over time, while individuals previously exposed to HSV tend to show dysregulated but more steady microbial profiles

We also observed that persistent HR-HPV tends to coincide with microbiome patterns linked to inflammation, pointing to a possible role of virus-anal microbiome interactions