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Effects of Substance Use and Sexual Behavior on the Intestinal Microbiome in HIV

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Background

- The intestinal microbiome helps maintain gut immune homeostasis, and perturbations affect mucosal barrier integrity and immune function
- Numerous factors influence microbiome composition including age, diet, and geography
- HIV-1 infection alters the intestinal microbiome; however, specific bacterial genera affected differ across studies suggesting that additional confounders may be present.
- This study sought to examine the effects of two potential confounders, substance use and sexual behavior, on the intestinal microbiome in HIV-infected men who have sex with men (MSM)

Methods

- Samples were obtained from an ongoing cohort (The mSTUDY) examining the effects of substance use on HIV-1 transmission and pathogenesis in young MSM
- Specimens: Rectal sponges, urine drug testing, and substance use and sexual behavior questionnaires were obtained from HIV+ participants (n=37) at baseline and six-month follow-up visits
- Microbiome: Microbiome profiling was performed using 16S rRNA gene sequencing and data processed using DADA2.
- Associations between substance use and sexual behavior and microbiota abundance were examined using zero-inflated negative binomial regression (ZINB)
- Further analysis of selected variable of interest was performed using propensity scores (PS) to account for multiple confounders

Results

Table 1. Study participant demographics and clinical characteristics, N=37

	Visit 1	Visit 2	p-value*
Age (years), mean (SD)	33.9 (6.5)		
Ethnicity, n (%)			
Black, non-Hispanic	17 (46)		
Black, Hispanic	1 (3)		
Hispanic, all races	17 (46)		
White, non-Hispanic	0 (0)		
Refuse to answer	2 (5)		
Employment, n (%)			
Disabled	11 (30)		
Unemployed	11 (30)		
Full time	5 (13)		
Part time	8 (22)		
Student	2 (5)		
Experienced homelessness in past six months, n (%)	12 (32)	7 (19)	0.06
Body mass index (BMI, kg/m^2), mean (SD)	26.1 (4.8)	26 (4.6)	0.87
Years since HIV diagnosis, mean (SD)	7.2 (7)		
HIV-1 RNA (copies/ml), mean (SD)	55,770 (105,488)	37,826 (88,454)	0.02
CD4+ T cells (cells/µl), mean (SD)	444 (255)	501 (247)	0.01
Currently on antiretroviral therapy (ART), n (%)	30 (81)	30 (81)	>0.99
ART regimen, n (%)			0.99
NNRTI	10 (27)	9 (24)	
PI	8 (22)	9 (24)	
INSTI	10 (27)	10 (27)	
NNRTI + INSTI	1 (3)	1 (3)	
PI + INSTI	1 (3)	1 (3)	
Sexually transmitted infection (STI) prevalence, n (%)			
Syphilis (RPR, rapid plasma reagin)	0	2 (5)	0.16
N. gonorrhoeae, rectal	3 (8)	4 (11)	0.66
N. gonorrhoeae, urine	0	0	
N. gonorrhoeae, pharyngeal	2 (5)	2 (5)	>0.99
C. trachomatis, rectal	5 (14)	2 (5)	0.08
C. trachomatis, urine	2 (5)	0	0.16
C. trachomatis, pharyngeal	1 (3)	1 (3)	>0.99

*p-values for change between visits were computed using the following tests: Binary variables were compared using McNemar's test, continuous variables using the Wilcoxon signed-rank test, ordinal variables using mixed ordinal logistic regression, and NxN (N>2) nominal variables using mixed multinominal logistic regression models

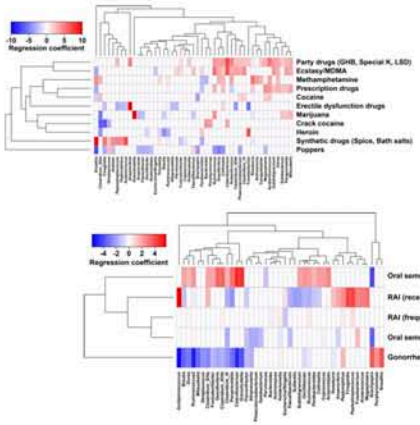


Figure 3. Associations between microbiome composition and substance use or sexual behavior. Heat map showing associations after controlling for CD4+ T cells and HIV-1 RNA copies. Red shading indicates positive associations and blue shading indicates negative associations. Intensity of shading refers to the relative strength of the association determined by regression coefficients from ZINB regression analysis.

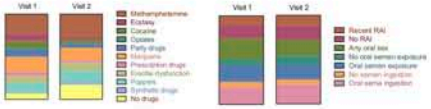


Figure 1. Self-reported substance use and sexual behavior among study participants. Stacked columns show distribution of substance use (left) and sexual behavior (right) per visit.

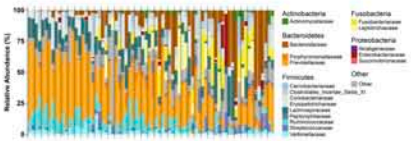


Figure 2. Taxonomic distribution and similarity between samples across time points. Stacked columns show relative abundance as a percentage of total bacterial sequences at the genus level. Vertical grey lines delineate different individuals.

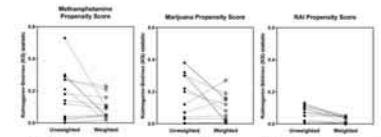


Figure 4. Propensity scores balance multiple confounders. PS were calculated to balance ethnicity, smoking, alcohol use, other substance use, HIV RNA copies, CD4+ T cells, and recent oral sex. Three PS were created to separately examine methamphetamine, marijuana, and receptive anal intercourse. Balance was assessed using Kolmogorov-Smirnov statistics; any variable with KS statistic >0.2 was included as a separate covariate.

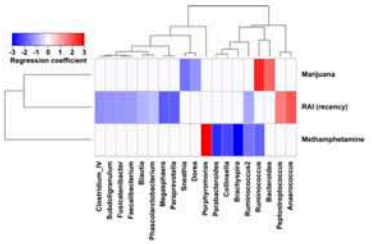


Figure 5. Propensity score-adjusted associations between microbiome and methamphetamine use, marijuana use, and receptive anal intercourse (RAI). Heat map showing adjusted associations after balancing of multiple confounders using PS. Red shading indicates positive associations and blue shading indicates negative associations from ZINB regression analysis.

Summary / Conclusions

- Both substance use and sexual behavior were associated with dysbiosis in HIV-infected MSM in this study
- Propensity-score adjusted analyses showed contrasting associations between methamphetamine and marijuana use and microbiome composition
- Methamphetamine use was associated with increased *Porphyromonas* and decreased *Ruminococcus* and *Parabacteroides*.
- Marijuana use was associated with increased *Ruminococcus* and *Bacteroides*
- Receptive anal intercourse (RAI) was specifically associated with increased *Peptostreptococcus* and *Anaerococcus*
- This study highlights the importance of considering behavioral factors in the interpretation and design of microbiome studies in HIV

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