Clinical Infectious Diseases

MAJOR ARTICLE

The Impact of Human Immunodeficiency Virus Infection on Gut Microbiota α-Diversity: An Individual-level Meta-analysis

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Background. Whether human immunodeficiency virus (HIV) infection impacts gut microbial α-diversity is controversial. We reanalyzed raw 16S ribosomal RNA (rRNA) gene sequences and metadata from published studies to examine α-diversity measures between HIV-uninfected (HIV^-) and HIV-infected (HIV^+) individuals.

Methods. We conducted a systematic review and individual level meta-analysis by searching Embase, Medline, and Scopus for original research studies (inception to 31 December 2017). Included studies reported 16S rRNA gene sequences of fecal samples from HIV^+ patients. Raw sequence reads and metadata were obtained from public databases or from study authors. Raw reads were processed through standardized pipelines with use of a high-resolution taxonomic classifier. The χ^2 test, paired *t* tests, and generalized linear mixed models were used to relate α-diversity measures and clinical metadata.

Results. Twenty-two studies were identified with 17 datasets available for analysis, yielding 1032 samples (311 HIV^-, 721 HIV^+). HIV status was associated with a decrease in measures of α-diversity ($P < .001$). However, in stratified analysis, HIV status was associated with decreased α-diversity only in women and in men who have sex with women (MSW) but not in men who have sex with men (MSM). In analyses limited to women and MSW, controlling for HIV status, women displayed increased α-diversity compared with MSW.

Conclusions. Our study suggests that HIV status, sexual risk category, and gender impact gut microbial community α-diversity. Future studies should consider MSM status in gut microbiome analyses.

Keywords. HIV; AIDS; microbiome.

Potent antiretroviral therapy (ART) has dramatically increased the lifespan of people infected with human immunodeficiency virus (HIV^+). Despite receiving effective ART, the average life expectancy of HIV^+ individuals remains lower than that of uninfected individuals (HIV^-) [1]. This appears to be driven by inflammation-related clinical diseases (eg, cardiovascular disease [CVD], stroke, cancer, long-bone fractures, and renal dysfunction), for which HIV^+ patients are disproportionately at risk [2–4]. While mechanisms are incompletely understood, recent studies raise the possibility that gut microbial dysbiosis contributes.

HIV infection promotes a chronic systemic proinflammatory state that is only partially reversed by ART-induced HIV viral load (VL) suppression [2]. Research suggests that, even with HIV virologic control, gut microbiome alterations, combined with decreased intestinal barrier function and increased bacterial translocation from the intestine, drive systemic inflammation, promoting CVD and other chronic complications of HIV disease [5–10]. However, causality remains speculative.

These findings are intriguing. Yet, the field is hampered by lack of consensus on what characterizes the gut microbiota in HIV^+ individuals and distinguishes the HIV^+ and HIV^- gut microbiota. Gut microbial α-diversity is of interest because increased diversity is generally considered a marker of health. In contrast, decreased diversity associates with several disease states (eg, obesity, inflammatory bowel disease, recurrent *Clostridioides difficile* infection) and predicts mortality in select populations (eg, hematopoietic stem cell recipients) [11–14].

Most studies in the HIV literature have compared α-diversity microbiome measures in HIV^+ and HIV^- subjects. However, studies have been heterogeneous (in sampled populations, sequencing techniques, and statistical analyses [including the measures of α-diversity examined]), often small in size, and, most importantly, have yielded inconsistent results. While a decrease in α-diversity is often associated with HIV [15–25], no

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difference [26–29] or even an increase [30] in fecal α-diversity measures in HIV$^+$ individuals is reported. Most studies did not control for sexual preference; only one examined sexual activity [21]. However, recent evidence suggests that status as a man who has sex with men (MSM) impacts gut microbiome measures, perhaps relating to receptive anal intercourse or other behaviors [17, 31–34]. Finally, limited data suggest that CD4 cell count [17, 24], HIV VL [17], and elite controller status [25] impact α-diversity in HIV$^+$ patients. The impact of ART on α-diversity is inconsistent [15, 18–20, 30, 35].

A more definitive understanding of α-diversity measures in HIV$^+$ compared to HIV$^-$ individuals may inform future studies examining the relationship between the gut microbiota and long-term complications (such as CVD, stroke, and cancer) in persons with HIV, as well as microbiota-based interventions to improve the health of these patients. Thus, herein, we conducted an individual level meta-analysis to identify differences in α-diversity in HIV$^+$ as compared with HIV$^-$ individuals using available 16S ribosomal RNA (rRNA) gene sequence data from published studies through December 2017. We reanalyzed these data using rigorous bioinformatics methods including a novel classification tool to define α-diversity measures and conducted stratified analyses incorporating key metadata such as gender, sexual orientation, and HIV treatment measures.

MATERIALS AND METHODS

Search Strategy and Selection Criteria

This was a systematic review and individual-level meta-analysis. Embase, Medline, and Scopus were searched with keyword and controlled vocabulary terms for HIV and the gastrointestinal microbiome (inception to 31 December 2017). Two independent reviewers (S. A. T., W. L. A. K.) assessed each article; differences were resolved by consensus. Unpublished data, reviews, studies lacking HIV$^+$ participants (exception, below), stool or rectal swab samples, 16S rRNA gene sequencing, and studies with <10 HIV$^+$ patients were excluded. Raw 16S rRNA gene sequence and metadata were downloaded from publicly available databases or obtained from study authors. In a sensitivity analysis, we incorporated stool samples from healthy HIV$^-$ individuals (no antibiotics for ≥3 months, immunosuppressants for the last month, and without a *C. difficile* diagnosis [ever]). Male and female pairs were matched on age and race [36] (see Supplementary Table 1 for search protocol).

Data Analysis

Raw 16S rRNA gene sequence data were preprocessed using one of 6 standardized protocols (Supplementary Table 2). In brief, paired-end 16S rRNA reads from the Illumina platform were merged into consensus sequences using FLASH [37] and filtered for quality and length using Trimmomatic [38] and QIIME [39, 40]. Sequences from the PhiX control genome were identified using BLASTN and removed. Roche/454 raw sequences were error corrected using ACACIA [41]. Passing sequences were trimmed of primers (when present), screened for chimeras using UCLUST (de novo mode) [42], and filtered for human-genome contaminant using Bowtie2 [43]. Chloroplast and mitochondrial contaminants were detected and filtered using the RDP classifier [44]. High-quality 16S rRNA gene sequences were assigned to a high-resolution taxonomic lineage using Resphera Insight [36, 45, 46], which generates a set of operational taxonomic units (OTUs) approximating the species-level composition of each sample. OTU profiles were rarified to an even level of coverage per sample for each study (mean, 9111 [range, 1000–20 000] reads; Supplementary Table 2); we intended to maximize the depth of coverage per sample while minimizing sample loss due to insufficient coverage. Meta-data variables including HIV status, age, race, gender, sexual orientation, ART use, CD4 count, and HIV VL were merged and reconciled between studies.

Four measures of α-diversity were calculated using QIIME. Observed species reports the total species observed and reflects sample richness [47]. Chao 1 also reflects richness, particularly in settings with many low-abundance classes [48]. It may not perform well in settings with low or different sequencing depths.[49]. In contrast, the Shannon index [50] and the inverse Simpson index [51] estimate both richness and evenness (Supplementary Table 3).

Generalized linear mixed models were used to relate raw and log-transformed α-diversity measures to clinical metadata. For all models, a random intercept for each study was included to account for study-specific variations in α-diversity. All *P* values were corrected for multiple comparisons through false discovery rate (FDR) across the 4 α-diversity measurements. Results in stratified analysis were validated with a nonparametric test (Wilcoxon rank sum). For regression coefficients to be comparable across α-diversity measurements, we divided the regression coefficients by their corresponding pooled standard deviation across all studies to generate stβ, which estimated effect size. To explore study-related heterogeneity, boxplots were generated using the ggplot2 package in R (version 3.3.0), after transformation of the α-diversity values through mean centering to zero (within each study) and scaling to unit variance. We additionally constructed Forest plots (requiring at least 5 patients/categories), using Hedge's G statistic, and calculated I^2 as a measure of heterogeneity for each subanalysis.

Sensitivity analyses removing the largest studies, studies with outlying results, studies conducted in non-European/non-US sites [19, 24, 29]; including HIV$^-$ samples from healthy women and men assumed to be heterosexual [36]; and including age in all models were conducted. Stata version 14 and R (version 3.3.0) software were utilized for all analyses. This research was deemed nonhuman subjects research by the Johns Hopkins Institutional Review Board (IRB00133905).

RESULTS

A total of 500 articles were identified after duplicate, erratum, and poster abstract removal (Figure 1). After review, 22 relevant articles were identified [15–22, 24–30, 35, 52–57]. We obtained data from 17 articles [15–21, 24, 25, 28–30, 52–55, 57] representing 17 separate datasets (5 datasets removed due to unavailable or incomplete data [22, 26, 27, 35, 56]) (Table 1 and Supplementary Table 4 present details of studies included and excluded, respectively). Due to low numbers of HIV^- individuals (particularly HIV^- men who have sex with women [MSW]), we obtained an additional 120 HIV^- samples (60 women and 60 men) [36]. These samples were not included in the main analysis; however, they were included in a sensitivity analysis as described above.

A total of 1032 individual samples were available, including samples from 311 HIV^- and 721 HIV^+ participants, including 114 HIV^- and 323 HIV^+ MSM (Supplementary Table 5). For all models, analyses were conducted with and without controlling for age; however, age did not significantly affect results (data not shown). All models were also run using log-transformed data (data not shown). Results did not change substantially over models using raw data; therefore, results were reported using raw data for ease of interpretation.

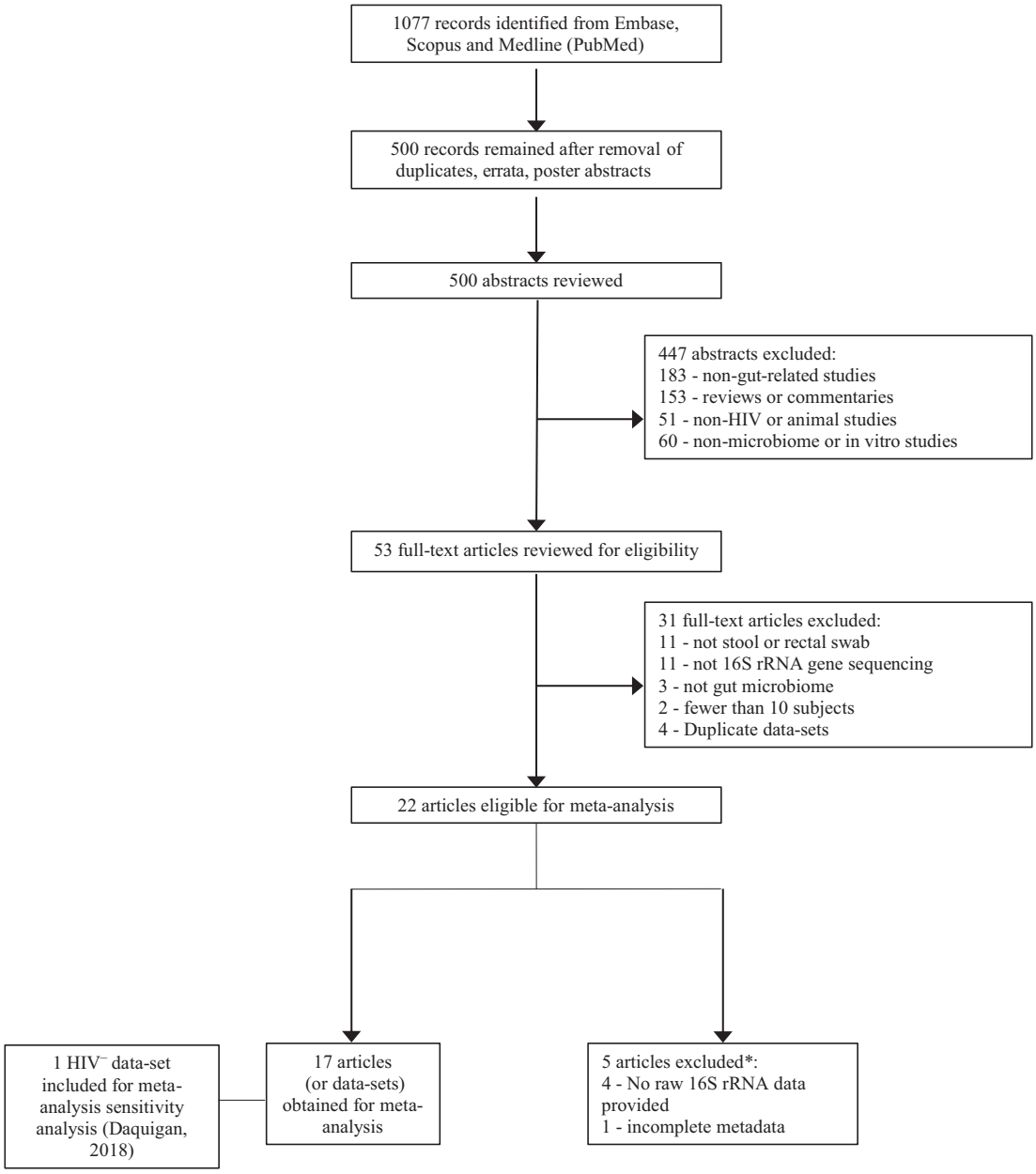

Figure 1. Summary of evidence search and selection. *See Supplementary Table 4. Abbreviations: HIV, human immunodeficiency virus; rRNA, ribosomal RNA.

Table 1. Characteristics of Studies Included

Author, Year [Ref]	Country	Study Period	Study Design	Study Setting	Study Population	Sample Type	16S rRNA Variable Region, Sequencing Platform	Diversity Measures[a]	Results: α-Diversity in HIV$^+$ c/w HIV$^-$	Limitations/ Potential Bias
Dillon 2014 [52]	US	ND	Cross-sectional study	University of Colorado Anschutz Medical Campus	18 HIV$^+$, 14 HIV$^-$	Rectal swabs	V4, Illumina MiSeq	ND	ND	Small sample size[b], lacked MSM[c]
Dinh 2015 [28]	US	ND	Prospective cohort	ID Clinic at Tufts Medical Center, Massachusetts	21 HIV$^+$, 16 HIV$^-$	Stool samples	V3–V5, Roche 454	Equitability, OTU, Shannon, Chao 1, PD	No significant differences.	Small sample size[b], lacked MSM[c]
Dubourg 2016 [15]	France	2012–2014	Case-control study	ID Departments in Conception and North Hospitals, Marseille	31 HIV$^+$, 27 HIV$^-$	Stool samples	V3–V4, Illumina MiSeq	Shannon	Decreased in HIV$^+$ c/w HIV$^-$.	Small sample size[b], lacked MSM[c]
Lozupone 2013 [30]	US	ND	Prospective cohort	University of Colorado Hospital ID Group Practice	25 HIV$^+$, 13 HIV$^-$	Rectal swabs	V4, Illumina MiSeq	OTU, Shannon, PD	Increased in HIV$^+$ untreated c/w HIV$^-$ (Shannon, PD).	Small sample size[b], lacked MSM[c]
Monaco 2016 [24]	Uganda	ND	Prospective cohort	Uganda AIDS Rural Treatment Outcomes study at Mbarara Regional Referral Hospital	82 HIV$^+$, 40 HIV$^-$	Stool samples	V4, Illumina MiSeq	Chao 1, PD	Near significant decrease (Chao 1) and significant decrease (PD) in HIV$^+$ CD4 <200 c/w HIV$^-$.	Lacked MSM[c]
Mutlu 2014 [16]	US	ND	Retrospective cohort	Tissue bank at Rush University Medical Center, Illinois	21 HIV$^+$, 22 HIV$^-$	Fecal sample from colon[d]	V1–V3, Roche 454	OTU, Chao 1, PD	Reduced in HIV$^+$ c/w HIV$^-$ (OTU, Chao 1, PD)	Small sample size[b], lacked MSM[c]
Noguera-Julian 2016 [17]	Spain, Sweden	2014	Cross-sectional study	HIV clinics at University Hospitals, community-based center.	Barcelona: 129 HIV$^+$, 27 HIV$^-$ Stockholm: 77 HIV$^+$, 7 HIV$^-$	Stool samples	V3–V4, Illumina MiSeq	OTU, Chao 1, ACE, Shannon, Simpson	Reduced in HIV$^+$ c/w HIV$^-$. MSM with increased diversity compared to non-MSM.	
Nowak 2015 [18]	Sweden	ND	Prospective cohort	HIV clinic at Karolinska University Hospital	31 HIV$^+$, 9 HIV$^-$	Stool samples	V3–V4, Illumina MiSeq	OTU, Shannon, Reciprocal Simpson	Reduced in HIV$^+$ c/w to HIV$^-$ by all measures.	Small sample size[b], lacked MSM[c]
Nowak 2017 [29]	Nigeria	ND	Cross-sectional study	Nested in the parent cohort (TRUST/ RV368)	75 HIV$^+$, 55 HIV$^-$	Rectal swabs	V3–V4, Illumina MiSeq	Shannon	No significant differences.	
Perez-Santiago 2013 [53]	US	ND	Prospective study	San Diego Primary Infection Cohort	13 HIV$^+$	Rectal swabs	V6, Illumina MiSeq	ND	No HIV$^-$ group.	Small sample size[b], no HIV$^-$
Pinto-Cardoso 2017 [19]	Mexico	ND	Cross-sectional study	National Institute of Respiratory Diseases, Mexico City	33 HIV$^+$, 10 HIV$^-$	Stool samples	V3–V4, Illumina MiSeq	OTU, Chao 1, PD, Shannon	Reduced in HIV$^+$ treated c/w HIV$^-$ by all measures.	Small sample size[b], lacked MSM[c]
Serrano-Villar 2017 [54]	Spain	ND	Prospective study	University Hospitals Clinico San Carlos and Ramón y Cajal	35 HIV$^+$, 9 HIV$^-$	Stool samples	V1–V3, Roche 454	ACE, Chao 1, OTU, Shannon	HIV$^+$ immune nonresponders less diverse c/w HIV$^-$ by all measures	Small sample size[b], lacked MSM[c]
Serrano-Villar 2017 [55]	Spain	ND	Cross-sectional study	High-resolution anoscopy clinic	42 HIV$^+$	Stool samples	V3–V4, Illumina MiSeq	Chao 1, Shannon	No HIV$^-$ group.	No HIV$^-$ controls[e]
Vesterbacka 2017 [25]	Sweden	ND	Cross-sectional study	HIV clinic at Karolinska University Hospital	48 HIV$^+$, 16 HIV$^-$	Stool samples	V3–V4, Illumina MiSeq	OTU, Chao 1, ACE, Shannon, Simpson	Reduced in HIV$^+$ untreated c/w HIV$^-$ (Chao 1, ACE, Shannon).	Few MSM[c]
Villanueva-Millan 2017 [20]	Spain	ND	Cross-sectional study	San Pedro's Hospital, Logrono	50 HIV$^+$, 21 HIV$^-$	Stool samples	V4, Illumina MiSeq	OTU, Chao1, α-index, Margalef diversity index	Reduced in HIV$^+$ c/w HIV$^-$ by all measures.	Lacked MSM[c]
Villar-Garcia 2017 [57]	Spain	Aug 2012–July 2013	Prospective study	Hospital del Mar Medical Research Institute	44 HIV$^+$	Stool samples	V3–V4, Illumina MiSeq	ND	ND	No HIV$^-$ controls

Table 1. Continued

Author, Year [Ref]	Country	Study Period	Study Design	Study Setting	Study Population	Sample Type	16S rRNA Variable Region, Sequencing Platform	Diversity Measures[a]	Results: α-Diversity in HIV$^+$ c/w HIV$^-$	Limitations/ Potential Bias
Yu 2014 [21]	US	1982–1999	Prospective study	Outpatient primary care clinics in Washington, DC and New York City	25 HIV$^+$, 51 HIV$^-$	Rectal swabs	V3–V4, Illumina MiSeq	OTU, Shannon, Chao 1, PD	Reduced in HIV$^+$ c/w HIV$^-$ by all measures; attenuated when adjusted for age, race, and smoking.	Small sample size[b]
Used only in sensitivity analysis										
Daquigan 2017 [36]	US	ND	Retrospective study	Multiple studies in US	120 HIV$^-$	Stool samples	V4	ND	ND	Lacked MSM[c]

Abbreviations: ACE, abundance-based coverage estimator; c/w, compared with; HIV$^-$, human immunodeficiency virus uninfected; HIV$^+$, human immunodeficiency virus infected; ID, infectious diseases; MSM, men who have sex with men; ND, not described; OTU, operational taxonomic unit; PD, phylogenetic diversity; rRNA, ribosomal RNA.

[a]See Supplementary Table 3.

[b]Thirty-five or fewer HIV$^+$ patients.

[c]Lacked information on MSM status, did not include MSM controls, or did not control for MSM status in analysis.

[d]Obtained during colonoscopy.

[e]Two additional HIV$^-$ patients were included in the dataset provided by authors.

HIV and α-Diversity: All Samples

HIV$^+$ patients were older (mean age, 40.9 years) than HIV$^-$ patients (mean age, 38.4 years) (P = .002). Before controlling for other factors, HIV status was strongly associated with a decrease in all measures of α-diversity, including observed species (FDR P < .0001, stβ = −0.48) and Chao 1 (FDR P < .0001, stβ = −0.38), Shannon (FDR P < .0001, stβ = −0.38) and inverse Simpson (FDR P = .001, stβ = −0.23) indices (Supplementary Table 6*A*). Forest plots of the 15 studies including both HIV$^+$ and HIV$^-$ samples (Figure 2A; Supplementary Figure 1*A*) and box plots (Figure 2B and 2C and Supplementary Figure 1*B* and 1*C*) show these same trends within most, though not all individual studies. Results were consistent in sensitivity analysis when an additional 120 HIV$^-$ samples [36] were added and after removal of the largest study [17], after removal of a study that was observed to be an outlier in mean observed species values [15], and after removal of the 3 non-European/non-US studies [19, 24, 29] (Supplementary Table 6*A*).

HIV and α-Diversity: MSM

When restricting the analysis to MSM only (n = 323 HIV$^+$ and n = 114 HIV$^-$), there was no significant association between HIV$^+$ status and α-diversity (observed species [FDR P = .377, stβ = −0.18], Chao 1 [FDR P = .548, stβ = −0.10], Shannon [FDR P = .377, stβ = −0.16], inverse Simpson [FDR P = .565, stβ = −0.07]). The results were fairly consistent across studies (see Forest plots in Figure 3A and Supplementary Figure 2 and boxplots in Supplementary Figure 3*A*–*D*). Results remained unchanged in sensitivity analysis after removal of the largest study [17] and the non-European/non-US studies [19, 29] (Supplementary Table 6*B*).

HIV and α-Diversity: Women

When restricting the analysis to women only (171 HIV$^+$ and 74 HIV$^-$), HIV$^+$ status was significantly associated with a decrease in α-diversity as measured by observed species (FDR P = .0001, stβ = −0.61), Chao 1 (FDR P = .0003, stβ = −0.55), and Shannon (FDR P = .0093, stβ = −0.40), with a trend toward a decreased diversity by inverse Simpson (FDR P = .130, stβ = −0.22). The results were fairly consistent across studies (Figure 3B, Supplementary Figures 3*A*–*D* and 4). Results were similar when additional HIV$^-$ samples [36] were added. HIV$^+$ status was associated with a decrease in all measures of α-diversity when the largest study [24] and the non-European/non-US studies [19, 24] were removed in sensitivity analysis (Supplementary Table 6*C*).

HIV and α-Diversity: MSW

When restricting the analysis to MSW (107 HIV$^+$ and 10 HIV$^-$) only, HIV$^+$ status was associated with a statistically significant decrease in observed species (FDR P = .036, stβ = −0.90), and a trend toward a decrease in Chao 1 (FDR P = .073, stβ = −0.66), Shannon (FDR P = .073, stβ = −0.68) and inverse Simpson (FDR P = .073, stβ = −0.61). Very few studies included HIV$^-$ MSW (Figure 3C, Supplementary Figures 3*A*–*D* and 5). In sensitivity

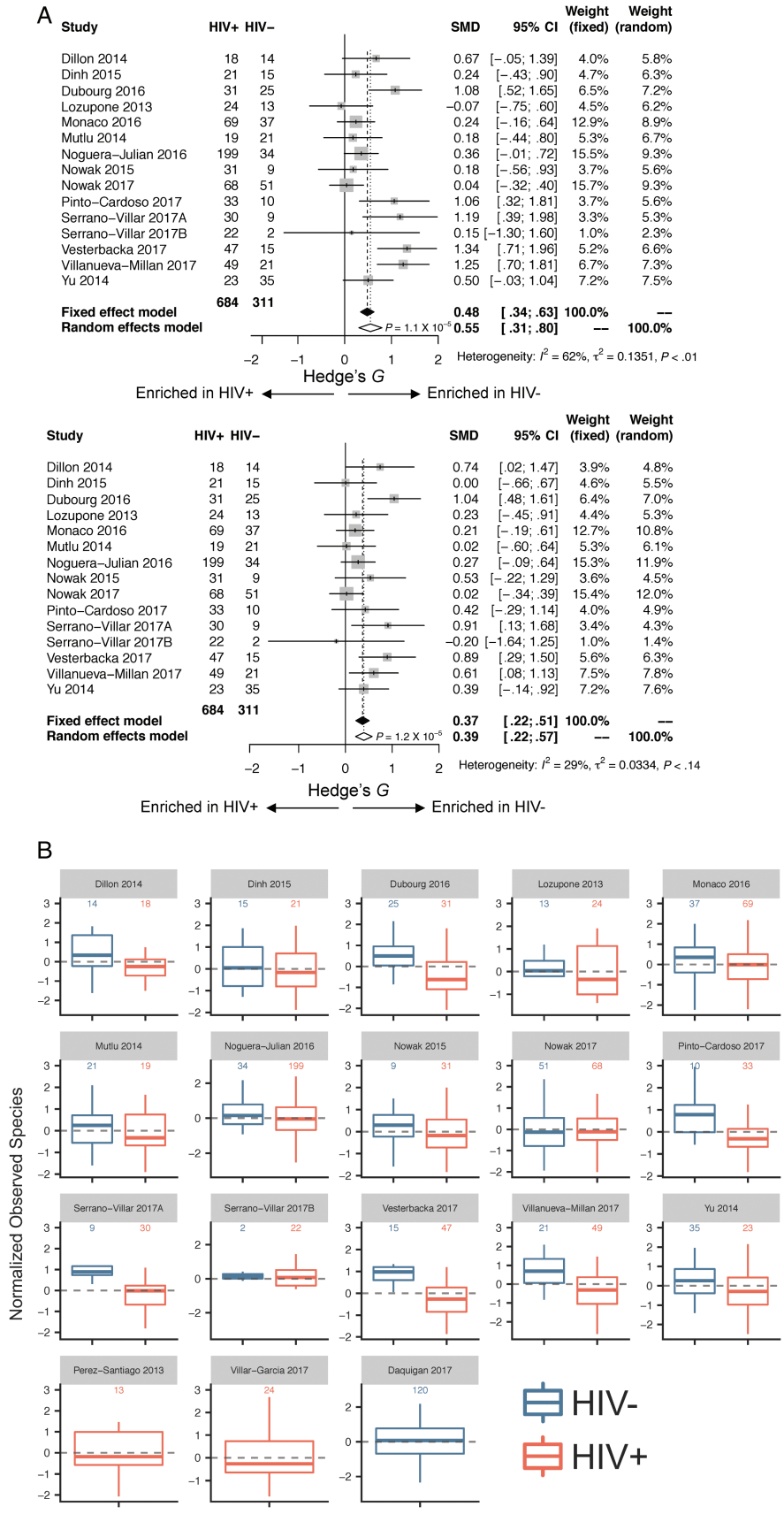

Figure 2. *A*, Forest plots utilizing all samples, comparing human immunodeficiency virus infected (HIV$^+$) to human immunodeficiency virus uninfected (HIV$^-$): observed species (above) and Shannon index (below). Associations between gut microbial α-diversity and HIV status. Hedge's *G* difference statistic is shown on the x-axis. Fixed

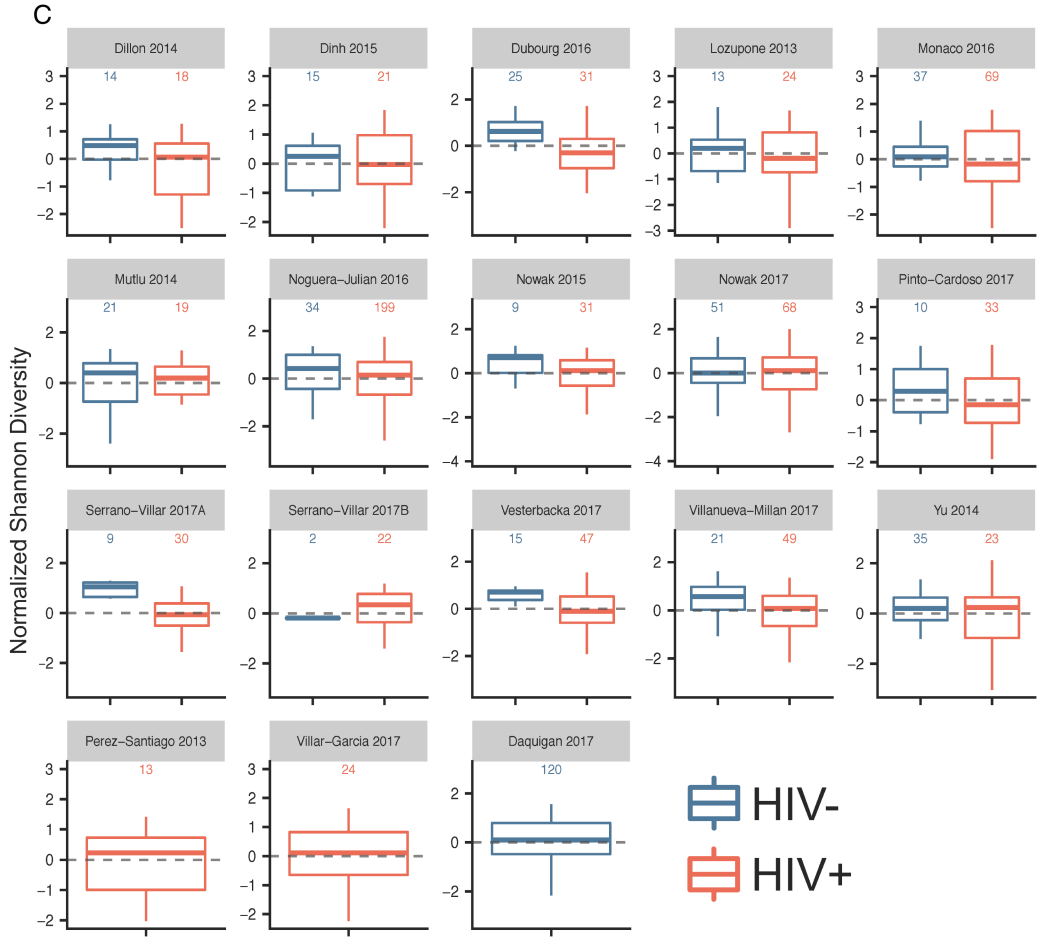

Figure 2. Continued

analysis, these results remained consistent when a non-European/non-US study [19] was removed. When the 60 additional (presumed MSW) HIV$^-$ men were added to the analysis, HIV$^+$ status was associated with a statistically significant decrease in all measures of diversity (Supplementary Table 6*D*).

HIV, Gender, and α-Diversity: Women and MSW

When restricting the analysis to MSW and women, HIV$^+$ status was associated with a statistically significant decrease in all measures of diversity (Supplementary Table 6*E*). When restricting to MSW and women but adjusting for gender and HIV, HIV$^+$ status remained statistically significantly associated with a decrease in all measures of diversity (observed species [FDR $P < .0001$, st$\beta = -0.70$], Chao 1 [FDR $P < .0001$, st$\beta = -0.59$], Shannon [FDR $P = .0004$, st$\beta = -0.48$], inverse Simpson [FDR $P = .020$, st$\beta = -0.30$]). Controlling for HIV status, heterosexual men had decreased diversity compared with women in terms of the Shannon (FDR $P = .0055$, st$\beta = -0.38$) and inverse Simpson (FDR $P = .005$, st$\beta = -0.40$) indices, with a trend toward decreased diversity in Chao 1 (FDR $P = .371$, st$\beta = -0.11$) and observed species (FDR $P = .172$, st$\beta = -0.19$). These results remained consistent when additional HIV$^-$ samples were added in sensitivity analysis (Supplementary Table 6*E*).

HIV$^+$ Individuals

Demographic, Clinical Factors, and HIV

Among HIV$^+$ participants, gender and, in men, sexual preference, were available for 601 individuals: 323 MSM, 107 MSW, and

effects models (black diamonds) and random effects models (white diamonds) with 95% CI above or below 0 were considered statistically significant. The fixed effects model assumes there exists a single effect size shared by all included studies, while the random effects model allows for variation in the effect size from study to study. Heterogeneity analysis includes estimates of I^2 (percentage of variation reflecting true heterogeneity), τ^2 (random effects between study variance), and *P* value from Cochran *Q* test for heterogeneity. Top panel of *A*: Based on observed species, gut microbial α-diversity is increased in HIV$^-$ as compared with HIV$^+$ patients. There is significant heterogeneity between studies ($I^2 = 62\%$, $P < .01$). Bottom panel of *A*: Based on Shannon index, gut microbial α-diversity is increased in HIV$^-$ as compared with HIV$^+$ patients. Heterogeneity between studies is not statistically significant ($I^2 = 29\%$, $P = .14$). *B* and *C*, Boxplots showing α-diversity in terms of observed species (*B*) and Shannon index (*C*) by study and HIV status (dark blue = HIV$^-$, red = HIV$^+$). α-Diversity is centered within study and scaled to unit variance. Most studies show decreased α-diversity in HIV$^+$ patients as compared with HIV$^-$ patients. Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; SMD, standardized mean difference.

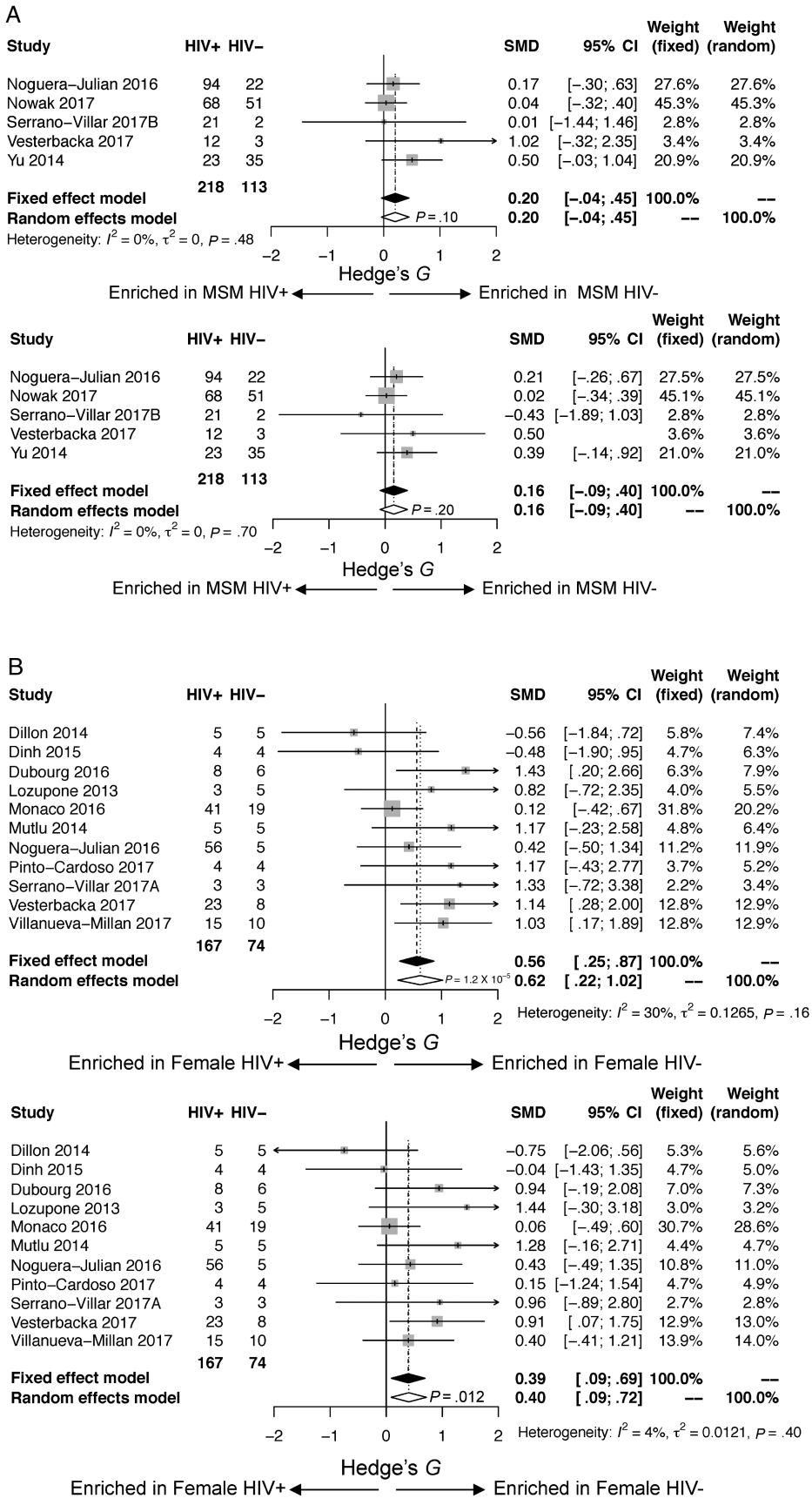

Figure 3. *A*, Forest plots restricted to men who have sex with men (MSM), comparing human immunodeficiency virus infected (HIV^+) to human immunodeficiency virus uninfected (HIV^-): observed species (above), Shannon (below). Associations between gut microbial α-diversity and HIV status in stratified analysis restricted to MSM. Hedge's

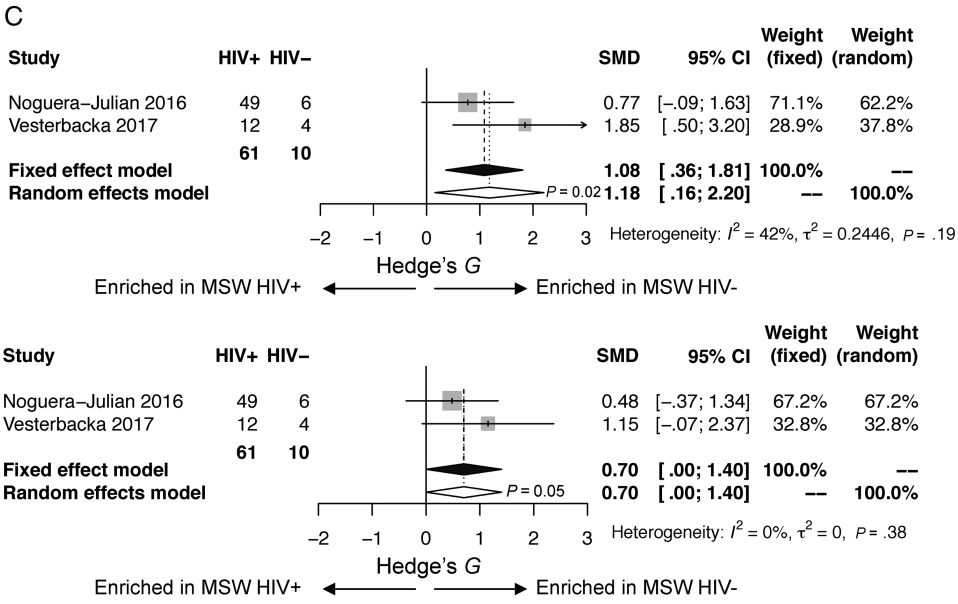

Figure 3. Continued

171 women. MSW were older than women, who were older than MSM. There were no significant differences in CD4 count between any of the groups in pairwise comparisons. However, MSM were more likely to have a detectable viral load (>400 copies/mL) than MSW and women. Women were more likely to have a detectable viral load than MSW. There was a trend toward MSM and MSW being more likely to be on ART than women (Table 2).

HIV, Demographic, Clinical Factors, and α-Diversity

Among HIV$^+$ individuals, controlling for MSM status and gender, CD4 count (dichotomized as <200 or >200 cells/μL), VL (dichotomized as <400 or >400 copies/mL), and ART use were not statistically significantly associated with α-diversity of observed species or Chao1. However, controlling for MSM status, HIV$^+$ men had decreased diversity (Shannon and inverse Simpson indices) compared with HIV$^+$ women (Table 3). In sensitivity analysis, removing non-European/non-US studies [19, 24, 29], results were largely similar, with a trend toward decreased Shannon diversity in HIV$^+$ men compared with HIV$^+$ women. Finally, when 2 studies [53, 54] were removed in which modest inconsistencies were noted between the published and provided data in terms of CD4 count and VL, results were unchanged (Supplementary Table 7*A*). In stratified analyses examining HIV$^+$ MSM only, ART use, CD4 count, and VL were not significantly associated with α-diversity. In a stratified analysis restricted to MSW, ART use was associated with decreased α-diversity as measured by observed species, Shannon, and inverse Simpson (Supplementary Table 7*B*).

G difference statistic is shown on the x-axis. Fixed effects models (black diamonds) and random effects models (white diamonds) with 95% confidence interval (CI) above or below 0 were considered statistically significant. The fixed effects model assumes there exists a single effect size shared by all included studies, while the random effects model allows for variation in the effect size from study to study. Heterogeneity analysis includes estimates of I^2 (percentage of variation reflecting true heterogeneity), τ^2 (random effects between study variance), and *P* value from Cochran *Q* test for heterogeneity. Top panel: Based on observed species, gut microbial α-diversity is not statistically significantly different in HIV$^-$ compared with HIV$^+$ MSM. There is little heterogeneity between studies (I^2 = 0%, *P* = .48). Bottom panel: Based on Shannon index, gut microbial α-diversity is not statistically significantly different in HIV$^-$ compared with HIV$^+$ MSM. There is little heterogeneity between studies (I^2 = 0%, *P* = .70). *B*, Forest plots restricted to women, comparing HIV$^+$ to HIV$^-$: observed species (above), Shannon (below). Associations between gut microbial α-diversity and HIV status in stratified analysis restricted to women. Hedge's *G* difference statistic is shown on the x axis. Fixed effects models (black diamonds) and random effects models (white diamonds) with 95% CI above or below 0 were considered statistically significant. The fixed effects model assumes there exists a single effect size shared by all included studies, while the random effects model allows for variation in the effect size from study to study. Heterogeneity analysis includes estimates of I^2 (percentage of variation reflecting true heterogeneity), τ^2 (random effects between study variance), and *P* value from Cochran *Q* test for heterogeneity. Top panel: Based on observed species, gut microbial α-diversity is increased in HIV$^-$ compared with HIV$^+$ women (*P* <.0001). There is little heterogeneity between studies (I^2 = 30%, *P* = .16). Bottom panel: Based on Shannon index, gut microbial α-diversity is increased in HIV$^-$ as compared with HIV$^+$ women (*P* = .012). There is little heterogeneity between studies (I^2 = 29%, *P* = .40). *C*, Forest plots restricted to men who have sex with women (MSW), comparing HIV$^+$ to HIV$^-$: observed species (above), Shannon (below). Associations between gut microbial α-diversity and HIV status in stratified analysis restricted to MSW. Hedge's *G* difference statistic is shown on the x-axis. Fixed effects models (black diamonds) and random effects models (white diamonds) with 95% CI above or below 0 were considered statistically significant. The fixed effects model assumes there exists a single effect size shared by all included studies, while the random effects model allows for variation in the effect size from study to study. Heterogeneity analysis includes estimates of I^2 (percentage of variation reflecting true heterogeneity), τ^2 (random effects between study variance), and *P* value from Cochran *Q* test for heterogeneity. Of note, there were only 10 HIV$^-$ MSW. Top panel: Based on observed species, gut microbial α-diversity is increased in HIV$^-$ compared with HIV$^+$ MSW (*P* = .02). There is little heterogeneity between studies (I^2 = 42%, *P* = .19). Bottom panel: Based on Shannon index, there is a trend toward gut microbial α-diversity being increased in HIV$^-$ compared with HIV$^+$ MSW (*P* = .05). There is little heterogeneity between the 2 studies (I^2 = 0%, *P* = .38).

Table 2. Summary Patient Characteristics of Included Studies

Characteristic	MSM	MSW	Women	Men With Unknown MSM Status	Unknown Gender, MSM Status	Statistical Significance (*P* Value)[a]		
						MSM vs MSW	MSM vs Women	MSW vs Women
Total HIV negative	114 (36.7)	10 (3.2)	74 (23.8)	104 (33.4)	9 (2.9)			
Age, y, mean (SD)	33.7 (11.0)	46.1 (5.7)	40.1 (9.3)[a]	42.1 (13.1)[b]	Unknown[c]	<.01	<.01	.05
Race								
White	53 (46.5)	8 (80.0)	30 (40.5)	29 (27.9)	0 (0.0)	.17	.19	.31
Black	51 (44.7)	2 (20.0)	24 (32.4)	27 (26.0)	0 (0.0)			
Latino	4 (3.5)	0 (0.0)	6 (8.1)	10 (9.6)	0 (0.0)			
Asian	0 (0.0)	0 (0.0)	1 (1.4)	2 (1.9)	0 (0.0)			
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			
Unknown	6 (5.3)	0 (0.0)	13 (17.6)	36 (34.6)	9 (100)			
Total HIV positive	323 (44.8)	107 (14.8)	171 (23.7)	89 (12.3)	31 (4.3)			
Age, y, mean (SD)	37.7 (11.3)[d]	48.3 (7.9)[e]	41.8 (10.6)[a]	40.9 (9.9)[f]	Unknown[c]	<.001	<.001	<.001
Race								
White	145 (44.9)	83 (77.6)	59 (34.5)	24 (27.0)	0 (0.0)	<.001	<.001	<.001
Black	74 (22.9)	12 (11.2)	80 (46.8)	32 (36.0)	0 (0.0)			
Latino	51 (15.8)	8 (7.5)	11 (6.4)	3 (3.4)	0 (0.0)			
Asian	0 (0.0)	0 (0.0)	4 (2.3)	1 (1.1)	0 (0.0)			
Other	1 (0.3)	1 (0.9)	2 (1.2)	1 (1.1)	0 (0.0)			
Unknown	52 (16.1)	3 (2.8)	15 (8.8)	28 (31.5)	31 (100)			
CD4 count, cells/μL								
<200	24 (7.4)	11 (10.3)	21 (12.3)	15 (16.9)	0 (0.0)	.36	.08	.61
≥200	297 (92.0)	96 (89.7)	150 (87.7)	74 (83.1)	0 (0.0)			
Unknown	2 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	31 (100)			
Viral load								
<400	157 (48.6)	71 (66.4)	91 (53.2)	46 (51.7)	0 (0.0)	.001	.28	.02
≥400	163 (50.5)	33 (30.8)	77 (45.0)	31 (35.8)	0 (0.0)			
Unknown	3 (0.9)	3 (2.8)	3 (1.8)	12 (13.5)	31 (100)			
ART use								
No	136 (42.1)	46 (43.0)	94 (55.0)	30 (33.7)	0 (0.0)	.68	.04	.05
Yes	164 (50.8)	61 (57.0)	77 (45.0)	59 (66.3)	0 (0.0)			
Unknown	23 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	31 (100)			

Data are presented as no. (%) unless otherwise indicated. *P* values represent χ^2 analysis. Based on Bonferroni correction, statistical significance set at *P* <.02.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; MSM, men who have sex with men; MSW, men who have sex with women; SD, standard deviation.

[a]Missing age on 5 individuals.

[b]Missing age on 16 individuals.

[c]Missing age on all individuals.

[d]Missing age on 25 individuals.

[e]Missing age on 1 individual.

[f]Missing age on 3 individuals.

[g]Excludes unknown categories.

DISCUSSION

Herein, we assembled the largest dataset available to date to evaluate α-diversity in the gut microbiome of HIV^+ compared within HIV^- individuals. Overall, HIV^+ status was significantly associated with decreased α-diversity, but only among MSW and women. When controlling for HIV, women had increased diversity as compared to MSW, consistent with recently presented data from another cohort [58]. Among HIV^+ individuals, we did not find overall associations with CD4 count, viral load, or ART use, albeit these parameters (along with gender and MSM status) were available in a more limited subset of individuals (79%). Taken together, our results imply that HIV status, gender, and sexual risk category impact α-diversity.

Reports of HIV-associated dysbiosis, including decreases in α-diversity, have raised significant interest in the development of microbiota-based interventions to alter the structure of the gut microbiota and improve the health of patients with HIV. In a recent small study, 6 HIV^+ individuals received fecal microbial transplantation (FMT) [59], and at least 2 clinical trials of FMT are planned, with the aim of improving dysbiosis and inflammation. Yet, as our study demonstrates, interactions between gender, MSM status, and HIV affect the microbiome and may significantly impact the outcome of such interventions.

There are significant limitations to our study. First, we were not able to obtain data from 5 studies. However, the majority of these studies were small (Supplementary Table 4). Second,

Table 3. Human Immunodeficiency Virus–infected Patients: Multivariate Model Including Men Who Have Sex With Men Status, Gender, CD4 Cell Count, Viral Load, and Antiretroviral Therapy Use

	Measures of α-Diversity			
Variable	Observed Species[a]	Chao 1[a]	Shannon[a]	Inverse Simpson[a]
All samples				
Men[b] (Ref: Women)	–0.18 (0.146)	–0.14 (0.233)	–0.34 (0.023)	–0.40 (0.011)
MSM (Ref: non-MSM[c])	0.15 (0.362)	0.15 (0.362)	0.10 (0.560)	–0.03 (0.800)
CD4 count (Ref: <200 cells/μL)	0.10 (0.594)	0.09 (0.594)	0.13 (0.594)	0.08 (0.594)
HIV viral load (Ref: <400 copies/mL)	–0.25 (0.143)	–0.16 (0.182)	–0.23 (0.143)	–0.24 (0.143)
ART use (Ref: no ART)	–0.19 (0.282)	–0.16 (0.282)	–0.14 (0.334)	–0.21 (0.282)

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; MSM, men who have sex with men.

[a]stβ (false discovery rate adjusted *P* value).

[b]Men includes MSM and men who have sex with women (MSW).

[c]Non-MSM includes women and MSW.

patient metadata were collected using different approaches (some retrospective, some prospective) for each study, and multiple studies did not collect the full set of characteristics of interest across all subjects. We attempted to reconcile these variables, but the heterogeneous ways in which metadata were collected could introduce bias and potential misclassification. We were unable to account for type or duration of ART, both of which could affect α-diversity. Importantly, increased diversity has been seen in gut microbiota of individuals living in agrarian African societies as compared to urban, European controls [60], with diet and antibiotic use further impacting the gut microbial composition [61, 62]; associations between race and the gut microbiota are less robust [63]. Populations in our study came from differing geographic regions, and unmeasured factors, such as diet, smoking, recent or distant antibiotic use, and race could have influenced results. To address this possibility, we conducted sensitivity analyses in which the largest studies and non-European/non-US studies were removed from our analyses, which led to broadly unchanged results.

Finally, cohort design, sample collection techniques, DNA extraction protocols, primer sets, and sequencing platforms differed between studies. We found (see Forest plots) that studies utilizing rectal swabs [21, 29, 30, 52, 53] fell within range of those using stool samples. Additionally, we designed 6 tailored preprocessing protocols that adapted to subtle differences in the underlying raw data with the goal of minimizing bias and maximizing high-quality sequences for downstream analysis. Sequence data from each study that passed preestablished quality metrics were subjected to a taxonomic assignment algorithm that prioritized classification of each individual sequence to avoid potential biases associated with 16S rRNA gene region and clustering based on sequence similarity. Importantly, to normalize within and across studies, we subsampled to an even level of coverage within each study prior to downstream α-diversity calculations and included a random effects term in our statistical models to account for study-to-study variation. However, despite our efforts, it remains possible that differing techniques at every stage (including choice of 16S rRNA gene [V] region; Table 1) could have introduced bias.

In these 17 datasets, only 10 samples from HIV$^-$ MSW were available for analysis, which limited power, particularly for stratified analyses restricted to MSW. To address this, we obtained samples from a study including only HIV$^-$ men and women [36]. Although information on MSM status was not available, we found that with inclusion of these samples in a sensitivity analysis, trends in Chao1, Shannon, and inverse Simpson indices, suggesting decreased α-diversity in the fecal samples of HIV$^+$ MSW as compared to HIV$^-$ MSW, became statistically significant.

Despite these limitations, our findings clarify and extend the reported findings regarding α-diversity in HIV$^+$ vs HIV$^-$ individuals. Within the prolific research on the microbiome, studies often report on results with small numbers of patient samples and use heterogeneous analytic techniques that together likely contribute to the conflicting results reported. Thus, one approach applicable, not only within HIV research, but also within the broader microbiome research field, is to try to resolve study differences by compiling and reanalyzing data in a standardized fashion, with the goal of identifying more definitive patterns. Results from our overall HIV$^+$ vs HIV$^-$ analysis were broadly consistent with the original study results (Figure 2A and Supplementary Figure 1*A*); however, compiling data enabled us to conduct stratified analyses, which revealed additional nuances. Our individual level meta-analysis, along with similar prior efforts by Drewes et al [64], illustrates the potential to refine knowledge using this approach and to inform future study design and research questions. Herein, we observed that gender and sexual risk category impact the relationship between HIV status and α-diversity. Future studies should collect and consider these variables in study designs to identify associations between clinical outcomes and gut microbiota features.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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