



## Article

# Comparison of the Fecal Bacteriome of HIV-Positive and HIV-Negative Older Adults

Matilde Sánchez-Conde <sup>1,2,\*</sup>, Claudio Alba <sup>3</sup>, Irma Castro <sup>3</sup>, Fernando Dronda <sup>1</sup>, Margarita Ramírez <sup>4</sup> , Rebeca Arroyo <sup>3</sup>, Santiago Moreno <sup>1</sup> , Juan Miguel Rodríguez <sup>3</sup> and Fátima Brañas <sup>5,\*</sup>

<sup>1</sup> Infectious Diseases Department, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), 28034 Madrid, Spain; fdronda.hrc@salud.madrid.org (F.D.); smguillen@salud.madrid.org (S.M.)

<sup>2</sup> CIBER de Enfermedades Infecciosas (CIBERINFECT), Instituto de Salud Carlos III, 28220 Madrid, Spain

<sup>3</sup> Department of Nutrition and Food Science, Complutense University of Madrid, 28040 Madrid, Spain; c.alba@ucm.es (C.A.); irmacastro@ucm.es (I.C.); jmrodrig@ucm.es (J.M.R.)

<sup>4</sup> Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain; ramirezarga@yahoo.es

<sup>5</sup> Geriatric Department, Hospital Universitario Infanta Leonor, 28031 Madrid, Spain

\* Correspondence: msconde@gmail.com (M.S.-C.); fbranas@gmail.com (F.B.)

**Abstract:** HIV infection is considered a scenario of accelerated aging. Previous studies have suggested a link between aging, frailty, and gut dysbiosis, but there is a knowledge gap regarding the HIV population. Our objective was to compare the fecal bacteriome of older people with HIV (PWH) and non-HIV controls, and to assess potential links between gut dysbiosis and frailty. A total of 36 fecal samples (24 from PWH and 12 from non-HIV controls) were submitted to a metataxonomic analysis targeting the V3–V4 hypervariable region of the 16S rRNA gene. High-quality reads were assembled and classified into operational taxonomic units. Alpha diversity, assessed using the Shannon index, was higher in the control group than in the HIV group ( $p < 0.05$ ). The relative abundance of the genus *Blautia* was higher in the HIV group ( $p < 0.001$ ). The presence of *Blautia* was also higher in PWH with depression ( $p = 0.004$ ), whereas the opposite was observed for the genus *Bifidobacterium* ( $p = 0.004$ ). Our study shows shifts in the composition of the PWH bacteriome when compared to that of healthy controls. To our knowledge, this is the first study suggesting a potential link between depression and gut dysbiosis in the HIV population.

**Keywords:** gut microbiome; HIV; aging; frailty; dysbiosis; depression



**Citation:** Sánchez-Conde, M.; Alba, C.; Castro, I.; Dronda, F.; Ramírez, M.; Arroyo, R.; Moreno, S.; Rodríguez, J.M.; Brañas, F. Comparison of the Fecal Bacteriome of HIV-Positive and HIV-Negative Older Adults. *Biomedicines* **2023**, *11*, 2305. <https://doi.org/10.3390/biomedicines11082305>

Academic Editor: Ryota Niikura

Received: 2 August 2023

Revised: 12 August 2023

Accepted: 16 August 2023

Published: 19 August 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

HIV infection has been postulated to be a model of accelerated aging due to the chronic activation of the immune system, even when a patient is undergoing optimal immunovirological control treatment. This immune activation originates from prolonged antigenic stimulation, concurrent infections, and a continuous translocation of gut-associated microbes [1]. Microbial translocation is favored by the HIV-associated impairment of the gut epithelial barrier and the alteration of the gut microbiome [2,3]. In turn, gut dysbiosis contributes to inflammation [4], further disrupting the epithelial barrier, exacerbating microbial translocation, and facilitating the progression of HIV infection [5]. The clinical expression is an increased prevalence of age-related non-HIV-associated comorbidities, including geriatric syndromes, and a rising prevalence of frailty occurring earlier than in the general population [6,7].

Frailty is closely associated with a worse clinical prognosis (e.g., falls, morbidity, or death), but there is the possibility of a successful outcome if it is detected at an early stage of the infection [8,9]. Among community-dwelling adults older than 65 years, the prevalence of frailty is approximately 7% [10–13], while among people with HIV (PWH),

it has been reported to be up to twice that for uninfected individuals who are 10 years older [14,15]. In fact, frailty is a clinical marker of age acceleration in both HIV and non-HIV populations [16–18]. Gut dysbiosis has been associated with inflammation in both older people and PWH [19], but there is a lack of information regarding older PWH in this field, which constitutes a knowledge gap [20–22].

As older PWH have an increased risk of frailty, a reversible condition with prognostic value, and may exhibit increased gut dysbiosis, our intention in this pilot study was to compare the composition of the fecal microbiome of PWH and that of non-HIV controls, as well as to assess if there are potential links with frailty in this population.

## 2. Materials and Methods

### 2.1. Study Design and Patients

A total of 36 fecal samples were analyzed in this work. Among them, 24 had been obtained from virologically suppressed PWH (>55 years old) from our frailty cross-sectional study [10], including 7 frail, 9 pre-frail, and 8 robust ones, matched for age and nadir CD4. The samples had been stored frozen (−80 °C) since then. In the frame of such a study, the population was screened for the prevalence of frailty and tested for physical function, depression, nutritional status, and associated factors [10]. In the present study, 12 healthy non-HIV people with a similar age distribution were also included as controls. The ethics committees of each hospital approved the study. All patients and controls signed informed consent forms.

### 2.2. Data Collection

Sociodemographic data, comorbidities (i.e., self-reported and physician-diagnosed chronic conditions), medications (i.e., polypharmacy was defined as taking  $\geq 6$  medications), and variables related to HIV infection (i.e., risk practice for HIV infection, the baseline and current immunovirologic status, and the stage of HIV infection at diagnosis) were recorded. Depression status was evaluated using the Short Geriatric Depression Scale (i.e., S-GDS or Yesavage test) [23]. Physical function was assessed by quantifying it using the Short Physical Performance Battery (SPPB) [24]. The recorded laboratory data included HIV-related data (i.e., HIV-RNA, nadir and current CD4 count, and CD4/CD8 rate).

### 2.3. Frailty

Frailty was assessed according to Fried's frailty phenotype, defined by 5 functional criteria [25], namely, shrinking (unintentional weight loss of  $\geq 4.5$  kg or  $\geq 5\%$  of body weight during the previous year), weakness (grip strength adjusted for gender and BMI), poor endurance and energy (self-reported exhaustion identified by 2 questions from the Center for Epidemiologic Studies Depression scale), slowness (based on the time to walk 4 m, adjusting for gender and standing height), and low physical activity level ( $< 383$  kcal/week in men and  $< 270$  kcal/week in women using the Minnesota Leisure Time Activity Questionnaire). Patients were considered frail when they met at least 3 of the 5 criteria, pre-frail when they met 1 or 2 criteria, and robust when they met no criteria.

### 2.4. Metataxonomic Analysis

A dual-barcoded 2-step PCR reaction was conducted to amplify a fragment of the V3–V4 hypervariable region of the bacterial 16S ribosomal RNA (rRNA) gene. Equimolar concentrations of the universal primers S-D-Bact-0341-b-S-17 (ACACTGACGACATG-GTTCTACACCTACGGGNGGCWGCAG) and S-D-Bact-0785-a-A-21 (TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC) were used. Barcodes used for Illumina sequencing were appended to the 3' and 5' terminal ends of the PCR amplicons to allow for the separation of forward and reverse sequences. A bioanalyzer (2100 Bioanalyzer, Agilent, Santa Clara, CA, USA) was used to determine the concentration of each sample. Barcoded PCR products from all samples were pooled at approximately equimolar DNA concentrations and run on a preparative agarose gel. The correctly sized band was excised

and purified using a QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) and then quantified with PicoGreen (BMG Labtech, Jena, Germany). Finally, 1 aliquot of pooled, purified, and barcoded DNA amplicons was sequenced using the Illumina MiSeq pair-end protocol (Illumina Inc., San Diego, CA, USA) at the facilities of the Scientific Park of Madrid (Spain). The sequences analyzed for this study are available in the BioSample database of the National Center for Biotechnology Information.

The amplified fragments and results were taxonomically analyzed using the Illumina™ software according to the manufacturer's guidelines and pipelines (version 2.6.2.3). The resulting high-quality reads were assembled and classified taxonomically into operational taxonomic units (OTUs) by comparison with the Illumina™ software according to the manufacturer's guidelines and pipelines (version 2.6.2.3) using a Bayesian classification method and a level of similarity of at least 97%.

The concentration of DNA in the 3 blank preparations was approximately 0.01 ng/μL. The decontam R package was used to identify, visualize, and remove contaminating DNA based on the DNA concentration in each sample.

### 2.5. Statistical Analysis

We used descriptive statistics to examine participant characteristics, which were expressed as frequency (percent) for categorical variables, mean ( $\pm$ SD) for normally distributed continuous variables, or median (interquartile range) for continuous variables with a skewed distribution.

We compared continuous variables using the t-test for independent variables. Then, we used the Wilcoxon and Mann–Whitney tests for variables with 2 factors and a non-normal distribution or when the group size was small, and the Kruskal–Wallis test was used with variables with 3 or more factors and a non-normal distribution. We assessed the association between qualitative variables using the chi-square test or the Fisher exact test when the groups were very small. In addition, we used linear regression to assess the differences in biological age between frail and robust patients, and we considered differences to be significant when  $p \leq 0.05$ . We used SPSS statistical package (version 20.0).

For bacteriome analysis, quantitative data were expressed as the median and interquartile range (IQR). We assessed differences between groups using Kruskal–Wallis tests and pairwise Wilcoxon rank sum tests to calculate comparisons between groups. Also, we made Bonferroni corrections to control multiple comparisons. We generated a table of amplicon sequence variants' OTU counts per sample and normalized the bacterial taxon abundances to the total number of sequences in each sample. Then, we studied alpha diversity using the Shannon diversity index with the R vegan package (version 2.5.6).

We used principal coordinate analysis (PCoA) to evaluate beta diversity and to plot patterns of bacterial community diversity through a distance matrix containing a dissimilarity value for each pairwise sample comparison. We performed quantitative (relative abundance) and qualitative (presence/absence) analyses using the Bray–Curtis index and binary Jaccard index, respectively. Then, we performed an analysis of variance of the distance matrices using the “nonparametric manova test” (PERMANOVA) adonis with 999 permutations, as implemented in the R vegan package, to reveal statistical significance. For multilevel pairwise adonis comparisons, we used the Holm–Bonferroni method for  $p$ -value correction using the “pairwiseAdonis” R package (version 0.0.1). We performed the linear discriminant analysis (LDA) and effect size (LEfSe) algorithms to predict those taxa that violate the null hypothesis of no difference between the control and PWH groups of patients. We performed this analysis with the online interface Galaxy [26].

## 3. Results

The main characteristics of the PWH patients are shown in Table 1.

**Table 1.** Main characteristics of the PWH patients and controls included in this study.

	All PWH N = 24	Frail-PWH N = 7	Non-Frail-PWH N = 17		Healthy Controls N = 12	p
			Prefrail N = 9	Robust N = 8		
Sex at birth (Women/Men)	4 W/20 M	2 W/5 M	2 W/7 M	0 W/8 M	4 W/8 M	
Age (years) Media (SD)	61.9 (7.6)	60 (3.9)	65.3 (10.9)	59.7 (4.2)	60.6 (6.1)	0.245
Years living with known HIV Media (SD)	16.5 (7.7)	19.8 (6.5)	17.5 (8.5)	12.5 (6.6)	-	0.164
CD4+ nadir cells/mm <sup>3</sup> Median (IQR)	170.6 (154)	171 (141.2)	116.6 (105.6)	231 (200)	-	0.325
Current CD4+ (cells/mm <sup>3</sup> ) Media (SD)	589.2 (342.1)	662.8 (579.4)	523.7 (207.8)	598.5 (191)	-	0.737
Rate CD4/CD8 Median (IQR)	0.87 (0.5)	0.70 (0.3)	0.74 (0.3)	1.1 (0.6)	-	0.124
Diagnosed with depression (N)	7	3	2	2	-	0.634
Polypharmacy * (N)	12	6	5	1	-	0.017
BMI kg/m <sup>2</sup> (N)						
<25	15	5	6	4	-	0.293
25–29	5	0	3	2		
>29	4	2	0	2		

\* Polypharmacy was defined as taking at least six or more comedications (excluding antiretroviral treatment).

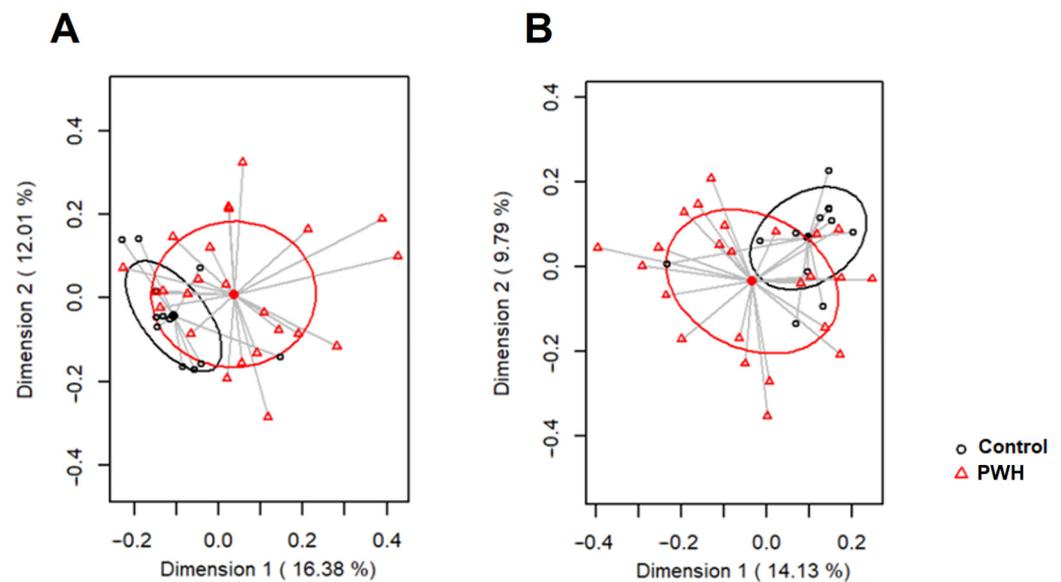
The sequencing of the 36 fecal samples yielded 5,829,212 high-quality reads (median = 168,180 reads/sample, ranging from 106,093 to 194,180), and, among them, a total of 347 OTUs were detected.

Initially, assessment of the alpha diversity using the Shannon index (median [IQR]) at the OTU level showed statistically significant differences between the PWH and the control groups. More specifically, the diversity of the latter group was significantly higher than the one found for the PWH group (3.74 [3.65–3.94] and 3.56 [3.32–3.69], respectively;  $p < 0.05$ ).

Next, both groups were compared for beta diversity. At the OTU level, the PCoA plots of the Bray–Curtis distance matrix (relative abundance) revealed that most of the samples clustered according to the HIV status ( $p = 0.012$ ; PERMANOVA test; Figure 1A). Similarly, the analysis according to the presence/absence of OTUs (binary Jaccard distance matrix) also revealed the existence of significant differences between both groups ( $p = 0.010$ ; Figure 1B).

A total of 16 phyla were observed in the fecal samples, with Firmicutes/Bacillota, Bacteroidetes/Bacteroidota, Proteobacteria/Pseudomonadota, and Actinobacteria/Actinomycetota being the most abundant ones. Although a correlation was not found between these phyla and the HIV status, a trend was observed toward a higher relative abundance of Bacteroidetes/Bacteroidota within the control group ( $p = 0.072$ ; Table 2).

Overall, a total of 135 bacterial genera were detected in this study. Some significant differences were found between both groups: the relative abundance of the genus *Blautia* was higher in the PWH group ( $p < 0.001$ ), whereas the opposite was found for the genera *Bacteroides*, *Oscillospira*, and *Clostridium* ( $p = 0.033$ ,  $p = 0.018$ , and  $p = 0.005$ , respectively; Table 2; Figure 2).



**Figure 1.** PCoA plots of bacterial profiles (at the OTUs level), based on the Bray–Curtis similarity analysis (A) and Jaccard's coefficient for binary data (B) from the fecal samples of control patients (control, black circles) and PWH patients (red triangles). The percentage of the total variance is explained by each axis.

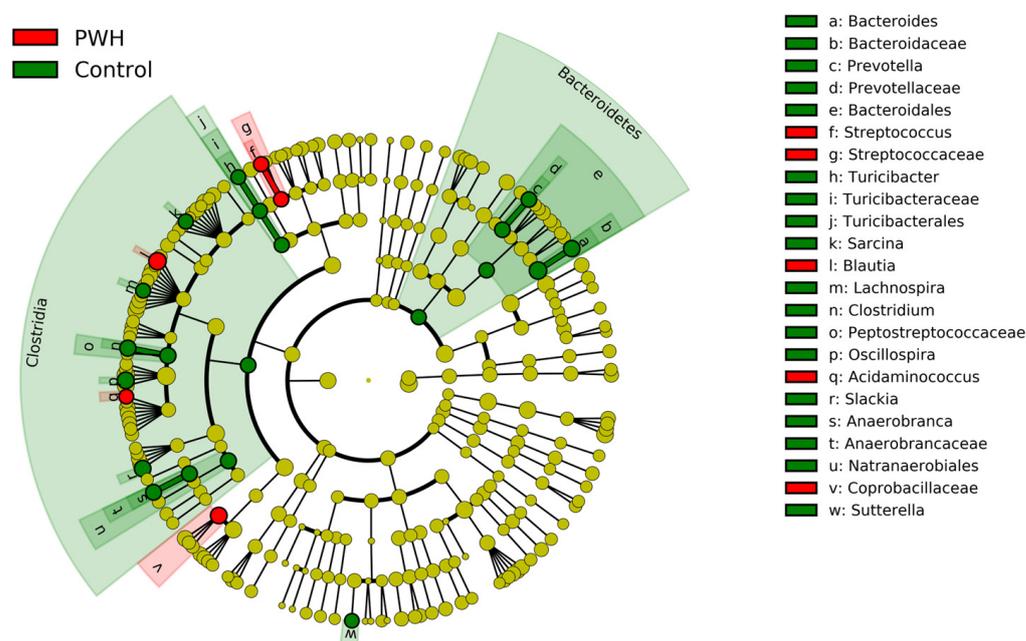
**Table 2.** Relative abundance (%), expressed as median and interquartile ranges, of the most abundant genera and phyla detected in the two groups (PWH and control) analyzed in this study.

Phyla/Genera	Control		PWH		p-Value *
	N (%) <sup>a</sup>	Median (IQR)	N (%)	Median (IQR)	
Firmicutes/Bacillota	12 (100%)	73.27 (67.71–77.6)	24 (100%)	74.51 (66.49–80.73)	0.730
<i>Faecalibacterium</i>	12 (100%)	9.25 (8.4–14.04)	24 (100%)	9.1 (5.07–15.04)	0.560
<i>Blautia</i>	12 (100%)	7.16 (5.82–8.48)	24 (100%)	11.18 (9.53–14.57)	<0.001
<i>Ruminococcus</i>	12 (100%)	9.99 (8.12–14.64)	24 (100%)	9.08 (6.42–11.71)	0.500
<i>Clostridium</i>	12 (100%)	5.49 (4.17–7.21)	24 (100%)	3.08 (2.42–3.72)	0.005
<i>Collinsella</i>	10 (83.33%)	2.59 (1.11–4.78)	21 (87.5%)	2.83 (0.67–7.56)	0.450
<i>Coprococcus</i>	12 (100%)	1.79 (0.79–3.13)	22 (91.67%)	2.54 (1.32–4.58)	0.180
<i>Slackia</i>	12 (100%)	3.23 (1.45–3.93)	23 (95.83%)	2.01 (0.38–3.88)	0.180
<i>Oscillospira</i>	12 (100%)	3.99 (2.5–4.49)	23 (95.83%)	1.32 (0.78–2.89)	0.018
<i>Alkaliphilus</i>	11 (91.67%)	3.44 (2.08–4.46)	22 (91.67%)	0.76 (0.33–2.13)	0.010
<i>Catenibacterium</i>	2 (16.67%)	<0.01 (<0.01–<0.01)	9 (37.5%)	<0.01 (<0.01–1.83)	0.210
<i>Roseburia</i>	12 (100%)	1.54 (0.86–2.49)	22 (91.67%)	1.21 (0.53–2.59)	0.700
<i>Eubacterium</i>	9 (75%)	0.57 (0.06–2.23)	17 (70.83%)	0.64 (<0.01–2.48)	0.970
<i>Erysipelothrix</i>	10 (83.33%)	0.55 (0.25–1.4)	24 (100%)	0.83 (0.39–1.65)	0.250
<i>Dorea</i>	12 (100%)	0.58 (0.35–0.71)	23 (95.83%)	0.9 (0.66–1.38)	0.022
Bacteroidetes/Bacteroidot:	12 (100%)	10.11 (6.6–13.5)	23 (95.83%)	7.31 (1.86–9.81)	0.072
<i>Bacteroides</i>	12 (100%)	5.89 (4.36–8.97)	21 (87.5%)	1.54 (0.5–6.61)	0.033
Proteobacteria/ Pseudomonota	12 (100%)	1.89 (1.45–5.5)	24 (100%)	1.55 (0.88–4.79)	0.560
<i>Escherichia</i>	8 (66.67%)	0.37 (<0.01–1.22)	13 (54.17%)	0.18 (<0.01–1.92)	0.740

Table 2. Cont.

Phyla/Genera	Control		PWH		p-Value *
	N (%) <sup>a</sup>	Median (IQR)	N (%)	Median (IQR)	
Actinobacteria/ Actinomycetota	12 (100%)	3.33 (1.36–6.68)	19 (79.17%)	1.29 (0.39–4.41)	0.320
<i>Bifidobacterium</i>	12 (100%)	3.21 (0.63–6.55)	15 (62.5%)	0.99 (<0.01–4.17)	0.240
Minor_phyla	9 (75%)	0.55 (<0.01–0.88)	20 (83.33%)	0.85 (0.3–2.23)	0.310
<i>Akkermansia</i>	4 (33.33%)	<0.01 (<0.01–0.25)	7 (29.17%)	<0.01 (<0.01–0.26)	0.950
Minor_genera	12 (100%)	13.87 (10.3–17.63)	24 (100%)	11.99 (8.66–19.25)	0.750
Unclassified_phyla	12 (100%)	7.21 (6.66–7.58)	24 (100%)	7.09 (6.46–7.66)	0.700
Unclassified_genera	12 (100%)	15.01 (13.96–15.5)	24 (100%)	12.22 (11.16–14.94)	0.210

<sup>a</sup>: Number of samples in which the phylum/genus was detected (relative frequency of detection). \* Wilcoxon rank test.



**Figure 2.** LefSe comparison between the abundance relative bacterial profile detected in the two groups (PWH [red] and control [green]) analyzed in this study.

When the sequences from the three PWH subgroups (i.e., frail, robust, and prefrail patients) were compared, no differences were found in relation to alpha and beta diversity or the taxonomic composition at the phyla and genera levels (Table 3).

Relative abundance of *Blautia* was higher among those PWH patients with depression (5.23 [13.96–16.99] vs. 10.38 [8.87–11.5];  $p = 0.004$ ), whereas the opposite was observed for the genus *Bifidobacterium* (3.07 [0.2–11.58] vs. <0.01 [<0.01–0.37],  $p = 0.022$ ). No differences were found among the patients of the PWH group when they were compared depending on frailty status, current and nadir CD4 status, CD4/CD8 ratio, years of HIV infection, body mass index, polypharmacy, and the remaining basal characteristics (Supplementary Table S1).

**Table 3.** Alpha and beta diversity and relative abundance (%), expressed as median and interquartile ranges, of the most abundant genera and phyla detected in the three PWH subgroups (robust, pre-frail, and frail) analyzed in this study.

	Robust (n = 8)		Pre-Frail (n = 9)		Frail (n = 7)		p-Value *
Shannon index	3.38 (3.28–3.65)		3.63 (3.51–3.7)		3.59 (3.37–3.62)		0.45
Bray–Curtis <sup>a</sup>	A		A		A		0.70 **
Jaccard <sup>a</sup>	A		A		A		0.19 **
Phylum/genera	N (%)	median (IQR)	N (%)	median (IQR)	N (%)	median (IQR)	
Firmicutes/Bacillota	8 (100%)	75.48 (66.12–82.3)	9 (100%)	70.64 (64.59–76.33)	7 (100%)	78.84 (69.33–81.75)	0.79
<i>Blautia</i>	8 (100%)	10.22 (8.31–12.12)	9 (100%)	10.81 (9.11–13.66)	7 (100%)	13.41 (11.39–15)	0.23
<i>Faecalibacterium</i>	8 (100%)	8.84 (3.43–12.44)	9 (100%)	10.59 (8.5–18.26)	7 (100%)	7.12 (4.12–15.45)	0.47
<i>Ruminococcus</i>	8 (100%)	9.08 (8.49–12.74)	9 (100%)	11.12 (4.77–11.7)	7 (100%)	8.7 (6.87–11.76)	0.88
<i>Collinsella</i>	7 (87.5%)	4.56 (0.63–10.24)	8 (88.89%)	2.69 (2.3–3.61)	6 (85.71%)	5.14 (1.61–7.71)	0.74
<i>Clostridium</i>	8 (100%)	2.96 (2.33–4)	9 (100%)	3.22 (2.98–3.63)	7 (100%)	2.77 (1.59–3.67)	0.47
<i>Coprococcus</i>	8 (100%)	1.96 (1.35–3.22)	9 (100%)	3.79 (2.27–4.08)	5 (71.43%)	2.22 (0.67–5.09)	0.60
<i>Slackia</i>	7 (87.5%)	2.57 (1.16–3.9)	9 (100%)	0.97 (0.46–2.88)	7 (100%)	2.12 (0.34–4.48)	0.79
<i>Catenibacterium</i>	4 (50%)	0.27 (<0.01–4.37)	2 (22.22%)	<0.01 (<0.01–<0.01)	3 (42.86%)	<0.01 (<0.01–1.89)	0.41
<i>Oscillospira</i>	8 (100%)	1.41 (0.77–2.37)	9 (100%)	1.59 (0.8–4.21)	6 (85.71%)	1.05 (0.8–2.49)	0.87
<i>Roseburia</i>	8 (100%)	0.84 (0.6–1.42)	9 (100%)	1.86 (1.29–3.27)	5 (71.43%)	1.14 (0.27–3.25)	0.56
<i>Eubacterium</i>	5 (62.5%)	1.51 (<0.01–4.01)	6 (66.67%)	0.27 (<0.01–1.3)	6 (85.71%)	1.3 (0.13–2.3)	0.71
<i>Erysipelothrix</i>	8 (100%)	0.8 (0.47–2.18)	9 (100%)	0.48 (0.36–0.78)	7 (100%)	1.45 (0.9–2.64)	0.13
<i>Alkaliphilus</i>	7 (87.5%)	0.42 (0.2–0.91)	9 (100%)	0.94 (0.58–1.52)	6 (85.71%)	1.58 (0.55–2.88)	0.22
<i>Dorea</i>	8 (100%)	1.4 (1.02–1.74)	9 (100%)	0.77 (0.64–0.85)	6 (85.71%)	0.89 (0.45–1.58)	0.08
Bacteroidetes/Bacteroidota	7 (87.5%)	5.84 (0.72–10.17)	9 (100%)	7.43 (5.96–8.95)	7 (100%)	2.91 (1.17–8.26)	0.42
<i>Bacteroides</i>	6 (75%)	1.05 (0.39–3.22)	9 (100%)	3.76 (1.55–6.59)	6 (85.71%)	0.44 (0.2–4.19)	0.16
Proteobacteria/Pseudomonodota	8 (100%)	1.55 (0.91–3.96)	9 (100%)	4.4 (1.32–9.24)	7 (100%)	1.42 (0.85–2.5)	0.38
<i>Escherichia</i>	4 (50%)	0.12 (<0.01–0.3)	5 (55.56%)	1.84 (<0.01–5.31)	4 (57.14%)	0.11 (<0.01–0.75)	0.62
Actinobacteria/Actinomycetota	5 (62.5%)	0.74 (<0.01–6.77)	8 (88.89%)	1.23 (0.63–3.4)	6 (85.71%)	3.74 (0.96–4.04)	0.79
<i>Bifidobacterium</i>	3 (37.5%)	<0.01 (<0.01–6.26)	6 (66.67%)	1.23 (<0.01–3.07)	6 (85.71%)	3.01 (0.47–3.72)	0.68
Minor_phyla	7 (87.5%)	0.68 (0.24–1.08)	7 (77.78%)	1 (0.43–2.22)	6 (85.71%)	0.72 (0.33–10.34)	0.79
<i>Akkermansia</i>	2 (25%)	<0.01 (<0.01–0.05)	2 (22.22%)	<0.01 (<0.01–<0.01)	3 (42.86%)	<0.01 (<0.01–8.1)	0.51
Minor_genera	8 (100%)	10.23 (7.57–14.83)	9 (100%)	11.91 (10.59–19.03)	7 (100%)	12.07 (9.1–18.79)	0.68
Unclassified_phyla	8 (100%)	7.33 (6.56–7.74)	9 (100%)	6.77 (6.45–7.17)	7 (100%)	7.26 (6.73–7.71)	0.46
Unclassified_genera	8 (100%)	12.38 (11.77–15.71)	9 (100%)	11.85 (11.21–13.19)	7 (100%)	12.33 (11.15–13.89)	0.88

<sup>a</sup>: No significant differences were observed among groups displaying the same letter. \* Kruskal–Wallis test. \*\* PERMANOVA test with 999 permutations.

#### 4. Discussion

In this study, we observed significant differences between the fecal microbiota of the PWH and control groups. This finding is congruent with those reported by other authors in previous studies, which showed that people living with HIV present alterations in the composition of their fecal microbiotas that are similar to those caused by aging [20,27,28], including decreased alpha diversity and lower abundance [29], which may be positively correlated with systemic inflammatory markers [19,30–32]. Correlations between gut dysbiosis, inflammation, and an increase in circulating biomarkers of gut epithelial barrier

damage and microbial translocation have been found in a nonhuman primate model of HIV infection [33]. These authors suggested that gut dysbiosis may maintain inflammation and metabolic alterations in chronic HIV patients despite proper long-term control of viremia.

The most abundant phyla identified in our study were Firmicutes/Bacillota, Bacteroidetes/Bacteroidota, Proteobacteria/Pseudomonadota, and Actinobacteria/Actinomycetota. HIV did not have a significant effect on the relative abundance of these phyla; however, the trend toward a lower abundance of the phylum Bacteroidetes in the PWH group is congruent with previously published data [28,34–36]. At the genus level, we detected 135 genera, and there were statistically significant differences between both groups in relation to the relative abundances of the genus *Blautia*, which was higher in the PWH group. *Blautia* is a commensal bacterial species that can become pathogenic, and, in fact, a higher abundance of this genus has been associated with some conditions, including irritable bowel syndrome, ulcerative colitis, and early breast cancer [37–39]. Previous studies have addressed the potential association between *Blautia* and HIV patients, and the results appear to be somehow controversial. So, while one study found that *Blautia* was highly relevant in HIV-infected individuals [40], another one found that this genus was enriched in naive HIV-infected patients but depleted in HIV-positive elite controllers and in HIV-negative individuals [41]. More recently, it has been described that *Blautia* sequences were more abundant in the colon and ileal samples collected by colonoscopy from HIV patients on antiretroviral therapy than in those from HIV-negative patients [42].

In addition, our results showed that the relative abundance of *Blautia* was higher in those PHW patients diagnosed with depression, whereas that of *Bifidobacterium* was higher in those who did not have depression. Although the etiopathogenesis of depression and other psychiatric disorders is still not completely elucidated, recent findings suggest that the dysbiosis of the gut microbiota might play a role in the severity of symptoms and, also, in modulating the efficacy and safety of treatments [43,44]. The relationship between depressive disorders and a higher abundance of *Blautia* and a lower one of *Bifidobacterium* in the fecal microbiome has already been reported [45–49], but, to our knowledge, this is the first study in which such potential associations have been linked to an HIV population. In other studies, changes in the fecal microbiome during the development of depressive-like behaviors in rats exposed to chronic unpredictable mild stress (CUMS) were assessed and compared with healthy controls [50]. Interestingly, the genus *Blautia* was reported to be more abundant in the CUMS group than in the healthy group. Interestingly, a higher abundance of *Blautia* has also been reported among HIV patients reporting distal neuropathic pain [29]. Although our study does not allow us to establish causality between depression and the presence of *Blautia* in HIV patients, this possibility should be the subject of future investigations.

On the other hand, we found that the abundance of the genera *Bacteroides* and *Oscillospira* was higher in the healthy control group. These findings are also consistent with previously published studies that have found significantly fewer *Bacteroides* in the PWH group than in the uninfected controls [28,30–32]. The decrease in the fecal abundance of some *Bacteroides* species, including *B. ovatus* and *B. thetaiotaomicron*, seems to be a feature of aging [51]. A significant reduction in the percentage of sequences belonging to this genus has also been detected in the context of other viral diseases, such as COVID-19, in patients when compared to healthy controls [52]. This fact may render the elderly population more vulnerable to COVID-19 since *Bacteroides* spp. have been associated with antiviral activity through a variety of mechanisms, including the downregulation of the expression of viral receptors and the alteration of receptor binding by their heparan sulfate-modifying glycosidase activities [53]. *Oscillospira* is a genus of commensal bacteria that produces butyrate and is commonly found in the gut of healthy hosts. Its abundance is negatively associated with metabolic syndrome-related parameters and with other diseases that involve inflammation [54,55]. However, the relationship between *Oscillospira* and HIV infection seems controversial since one report showed an abundance of this genus among HIV-positive

women in comparison with healthy controls [56], while others revealed that this genus was more abundant in HIV-positive elite controllers than in naive HIV-infected patients [41].

Finally, another relevant finding of our study is the absence of differences in the composition of the microbiota when the different subgroups of HIV-infected patients were compared according to their frailty status (i.e., frail, robust, or pre-frail). So far, data from the literature focused on the possible relationship between frailty and dysbiosis are inconclusive. While some studies have observed a lower abundance of butyrate-producing bacteria in frail patients, scientific evidence is limited regarding microbiome-related biomarkers, enabling a clear differentiation between frail and non-frail patients [57–61].

The main limitation of our study is the small sample size, which may be one of the reasons we did not find differences between the frail PWH and control groups or between polypharmacy and non-polypharmacy patients. Multicenter studies with larger cohorts are required to obtain more conclusive data in this field. Another limitation could be the fact that we do not have an in-depth analysis of all the foods in the patients' diet. However, there were no baseline differences, including years of HIV or BMI; none of the participants had received antibiotics in the previous 3 months; none of the participants had major dietary restrictions (e.g., vegan or vegetarian); and all were matched for age and sex at birth and came from the same geographical area.

## 5. Conclusions

In conclusion, our study further suggests the existence of an alteration of the fecal bacteriome in older adults with HIV when compared to healthy controls, a fact that may lead to the development of future strategies for modulating the gut microbiome of HIV patients. The altered gut bacteriome associated with HIV infection is not restored with correct antiretroviral therapy [62]. This fact may affect therapy outcomes since gut microbes and their metabolites play key roles in the development and regulation of host immunity [63]. As stated by Li et al. [62], understanding the factors shaping the so-called HIV-associated microbiome seems critical for developing novel approaches and therapies to improve the health of HIV patients.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biomedicines11082305/s1>, Table S1: Alpha and beta diversity in the fecal samples of the patients of the PWH groups according to other variables assessed in this study.

**Author Contributions:** M.S.-C., F.B. and J.M.R. designed and directed the project; M.S.-C., F.B., M.R., F.D. and S.M. recruited the patients and collected the data and the samples; I.C. and R.A. performed the experiments; C.A. performed the bioinformatic analysis; all authors contributed to the writing of the article or to its critical revision. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Clinical Research Ethics Committee of the Hospital Universitario Ramón y Cajal (Madrid, Spain). Protocol code 144/14 (data of approval 5 June 2014).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** We acknowledge all study participants who made this research possible.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Deeks, S.G. HIV infection, inflammation, immunosenescence, and aging. *Annu. Rev. Med.* **2011**, *62*, 141–155. [[CrossRef](#)]
2. Dillon, S.M.; Frank, D.N.; Wilson, C.C. The gut microbiome and HIV-1 pathogenesis: A two-way street. *AIDS* **2016**, *30*, 2737–2751. [[CrossRef](#)]
3. Ishizaka, A.; Koga, M.; Mizutani, T.; Parbie, P.K.; Prawisuda, D.; Yusa, N.; Sedohara, A.; Kikuchi, T.; Ikeuchi, K.; Adachi, E.; et al. Unique gut microbiome in HIV Patients on antiretroviral therapy (ART) suggests association with chronic inflammation. *Microbiol. Spectr.* **2021**, *9*, e0070821. [[CrossRef](#)] [[PubMed](#)]
4. Shirazi, M.S.R.; Al-Alo, K.Z.K.; Al-Yasiri, M.H.; Lateef, Z.M.; Ghasemian, A. Microbiome dysbiosis and predominant bacterial species as human cancer biomarkers. *J. Gastrointest. Cancer* **2020**, *51*, 725–728. [[CrossRef](#)] [[PubMed](#)]
5. Zevin, A.S.; McKinnon, L.; Burgener, A.; Klatt, N.R. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. *Curr. Opin. HIV AIDS* **2016**, *11*, 182–190. [[CrossRef](#)] [[PubMed](#)]
6. Guaraldi, G.; Orlando, G.; Zona, S.; Menozzi, M.; Carli, F.; Garlassi, E. Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin. Infect. Dis.* **2011**, *53*, 1120–1126. [[CrossRef](#)]
7. Greene, M.; Justice, A.C.; Covinsky, K.E. Assessment of geriatric syndromes and physical function in people living with HIV. *Virulence* **2016**, *8*, 586–598. [[CrossRef](#)] [[PubMed](#)]
8. Liu, C.K.; Fielding, R.A. Exercise as an intervention for frailty. *Clin. Geriatr. Med.* **2011**, *27*, 101–110. [[CrossRef](#)]
9. Bray, N.W.; Smart, R.R.; Jakobi, J.M.; Jones, G.R. Exercise prescription to reverse frailty. *Appl. Physiol. Nutr. Metab.* **2016**, *41*, 1112–1116. [[CrossRef](#)] [[PubMed](#)]
10. Abizanda, P.; Sánchez-Jurado, P.M.; Romero, L.; Paterna, G.; Martínez-Sánchez, E.; Atienzar-Núñez, P. Prevalence of frailty in a Spanish elderly population: The Frailty and Dependence in Albacete Study. *J. Am. Geriatr. Soc.* **2011**, *59*, 1356–1359. [[CrossRef](#)]
11. Santos-Eggimann, B.; Cuenoud, P.; Spagnoli, J.; Junod, J. Prevalence of frailty in middle-aged and older community-dwelling Europeans living in 10 countries. *J. Gerontol. A Biol. Sci. Med. Sci.* **2009**, *64*, 675–681. [[CrossRef](#)]
12. Danon-Hersch, N.; Rodondi, N.; Spagnoli, J.; Santos-Eggimann, B. Prefrailty and chronic morbidity in the youngest old: An insight from the Lausanne cohort Lc65+. *J. Am. Geriatr. Soc.* **2012**, *60*, 1687–1694. [[CrossRef](#)] [[PubMed](#)]
13. Macklai, N.S.; Spagnoli, J.; Junod, J.; Santos-Eggimann, B. Prospective association of the SHARE-operationalized frailty phenotype with adverse health outcomes: Evidence from 60+ community-dwelling Europeans living in 11 countries. *BMC Geriatr.* **2013**, *13*, 3. [[CrossRef](#)] [[PubMed](#)]
14. Brañas, F.; Jiménez, Z.; Sánchez-Conde, M.; Dronda, F.; De Quirós, J.C.L.B.; Pérez-Elías, M.J.; Miralles, P.; Ramírez, M.; Moreno, A.; Berenguer, J.; et al. Frailty and physical function in older HIV-infected adults. *Age Ageing* **2017**, *46*, 522–526. [[CrossRef](#)] [[PubMed](#)]
15. Brothers, T.D.; Kirkland, S.; Guaraldi, G.; Falutz, J.; Theou, O.; Johnston, B.L.; Rockwood, K. Frailty in people aging with human immunodeficiency virus (HIV) infection. *J. Infect. Dis.* **2014**, *210*, 1170–1179. [[CrossRef](#)] [[PubMed](#)]
16. Sánchez-Conde, M.; Rodríguez-Centeno, J.; Dronda, F.; López, J.C.; Jiménez, Z.; Berenguer, J.; Pérez-Elías, M.J.; Moreno, S.; Rodés, B.; Brañas, F. Frailty phenotype: A clinical marker of age acceleration in the older HIV-infected population. *Epigenomics* **2019**, *11*, 501–509. [[CrossRef](#)]
17. Desquilbet, L.; Margolick, J.B.; Fried, L.P.; Phair, J.P.; Jamieson, B.D.; Holloway, M.; Jacobson, L.P.; Multicenter AIDS Cohort Study. Relationship between a frailty-related phenotype and progressive deterioration of the immune system in HIV-infected men. *J. Acquir. Immune Defic. Syndr.* **2009**, *1*, 299–306. [[CrossRef](#)]
18. Weidner, C.; Lin, Q.; Koch, C.; Eisele, L.; Beier, F.; Ziegler, P.; Bauerschlag, D.O.; Jöckel, K.H.; Erbel, R.; Mühleisen, T.W.; et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol.* **2014**, *15*, R24. [[CrossRef](#)]
19. Vázquez-Castellanos, J.F.; Serrano-Villar, S.; Latorre, A.; Artacho, A.; Ferrus, M.L.; Madrid, N.; Vallejo, A.; Sainz, T.; Martínez-Botas, J.; Ferrando-Martínez, S.; et al. Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. *Mucosal Immunol.* **2015**, *8*, 760–772. [[CrossRef](#)]
20. Dillon, S.M.; Lee, E.J.; Kotter, C.V.; Austin, G.L.; Dong, Z.; Hecht, D.K.; Gianella, S.; Siewe, B.; Smith, D.M.; Landay, A.L.; et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunol.* **2014**, *7*, 983–994. [[CrossRef](#)]
21. Buford, T.W. (Dis)Trust your gut: The gut microbiome in age-related inflammation, health, and disease. *Microbiome* **2017**, *5*, 80. [[CrossRef](#)] [[PubMed](#)]
22. Biagi, E.; Nylund, L.; Candela, M.; Ostan, R.; Bucci, L.; Pini, E.; Nikkila, J.; Monti, D.; Satokari, R.; Franceschi, C.; et al. Through ageing, and beyond: Gut microbiota and inflammatory status in seniors and centenarians. *PLoS ONE* **2010**, *5*, e10667. [[CrossRef](#)]
23. Burke, W.J.; Wengel, R.W.H. The short form of the Geriatric Depression Scale: A comparison with the 30-item form. *J. Geriatr. Psychiatry Neurol.* **1991**, *4*, 173–178. [[CrossRef](#)] [[PubMed](#)]
24. Guralnik, J.M.; Simonsick, E.M.; Ferrucci, L.; Glynn, R.J.; Berkman, L.F.; Blazer, D.G.; Scherr, P.A.; Wallace, R.B. A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. *J. Gerontol.* **1994**, *49*, M85–M94. [[CrossRef](#)] [[PubMed](#)]
25. Fried, L.P.; Tangen, C.M.; Walston, J.; Newman, A.B.; Hirsch, C.; Gottdiener, J.; Seeman, T.; Tracy, R.; Kop, W.J.; Burke, G.; et al. Frailty in older adults: Evidence for a phenotype. *J. Gerontol. Mar.* **2001**, *56*, M146–M157. [[CrossRef](#)] [[PubMed](#)]
26. Afgan, E.; Baker, D.; Batut, B.; van den Beek, M.; Bouvier, D.; Cech, M.; Chilton, J.; Clements, D.; Coraor, N.; Grünig, B.A.; et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res.* **2018**, *46*, W537–W544. [[CrossRef](#)]

27. Erlandson, K.M.; MaWhinney, S.; Wilson, M.; Gross, L.; McCandless, S.A.; Campbell, T.B.; Kohrt, W.M.; Schwartz, R.; Brown, T.T.; Jankowski, C.M. Physical function improvements with moderate or high-intensity exercise among older adults with or without HIV infection. *AIDS* **2018**, *32*, 2317–2326. [[CrossRef](#)]
28. Mutlu, E.A.; Keshavarzian, A.; Losurdo, J.; Swanson, G.; Siewe, B.; Forsyth, C.; French, A.; Demarais, P.; Sun, Y.; Koenig, L.; et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. *PLoS Pathog.* **2014**, *10*, e1003829.
29. Ellis, R.J.; Heaton, R.K.; Gianella, S.; Rahman, G.; Knight, R. Reduced gut microbiome diversity in people with HIV who have distal neuropathic pain. *J. Pain* **2022**, *23*, 318–325. [[CrossRef](#)]
30. Tuddenham, S.A.; Koay, W.L.A.; Zhao, N.; White, J.R.; Ghanem, K.G.; Sears, C.L. The Impact of human immunodeficiency virus infection on gut microbiota alpha-diversity: An individual-level meta-analysis. *Clin. Infect. Dis.* **2020**, *70*, 615–627. [[CrossRef](#)]
31. Dinh, D.M.; Volpe, G.E.; Duffalo, C.; Bhalchandra, S.; Tai, A.K.; Kane, A.V.; Wanke, C.A.; Ward, H.D. Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. *J. Infect. Dis.* **2015**, *211*, 19–27. [[CrossRef](#)]
32. Monaco, C.L.; Gootenberg, D.B.; Zhao, G.; Handley, S.A.; Ghebremichael, M.S.; Lim, E.S.; Lankowski, A.; Baldridge, M.T.; Wilen, C.B.; Flagg, M.; et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. *Cell Host Microbe* **2016**, *19*, 311–322. [[CrossRef](#)]
33. Tanes, C.; Walker, E.M.; Slisarenko, N.; Gerrets, G.L.; Grasperge, B.F.; Qin, X.; Jazwinski, S.M.; Bushman, F.D.; Bittinger, K.; Rout, N. Gut microbiome changes associated with epithelial barrier damage and systemic inflammation during antiretroviral therapy of chronic SIV infection. *Viruses* **2021**, *13*, 1567. [[CrossRef](#)]
34. Dillon, S.M.; Wilson, C.C. What is the collective effect of aging and HIV on the gut microbiome? *Curr. Opin. HIV AIDS* **2020**, *15*, 94–100. [[CrossRef](#)] [[PubMed](#)]
35. Lozupone, C.A.; Li, M.; Campbell, T.B.; Flores, S.C.; Linderman, D.; Gebert, M.J.; Knight, R.; Fontenot, A.P.; Palmer, B.E. Alterations in the gut microbiota associated with HIV-1 infection. *Cell Host Microbe* **2013**, *14*, 329–339. [[CrossRef](#)] [[PubMed](#)]
36. Yang, L.; Poles, M.A.; Fisch, F.S.; Ma, Y.G.; Nossa, C.; Phelan, J.A.; Pei, Z. HIV-induced immunosuppression is associated with colonization of the proximal gut by environmental bacteria. *AIDS* **2016**, *30*, 19–29. [[CrossRef](#)]
37. Rajilić-Stojanović, M.; Biagi, E.; Heilig, H.G.; Kajander, K.; Kekkonen, R.A.; Tims, S.; de Vos, W.M. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* **2011**, *141*, 1792–1801. [[CrossRef](#)]
38. Nishino, K.; Nishida, A.; Inoue, R.; Kawada, Y.; Ohno, M.; Sakai, S.; Inatomi, O.; Bamba, S.; Sugimoto, M.; Kawahara, M.; et al. Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. *J. Gastroenterol.* **2018**, *53*, 95–106. [[CrossRef](#)] [[PubMed](#)]
39. Luu, T.H.; Michel, C.; Bard, J.M.; Dravet, F.; Nazih, H.; Bobin-Dubigeon, C. Intestinal proportion of *Blautia* sp. is associated with clinical stage and histoprognostic grade in patients with early-stage breast cancer. *Nutr. Cancer* **2017**, *69*, 267–275. [[CrossRef](#)]
40. San-Juan-Vergara, H.; Zurek, E.; Ajami, N.J.; Mogollon, C.; Pena, M.; Portnoy, I.; Vélez, J.I.; Cadena-Cruz, C.; Diaz-Olmos, Y.; Hurtado-Gómez, L.; et al. A Lachnospiraceae-dominated bacterial signature in the fecal microbiota of HIV-infected individuals from Colombia, South America. *Sci. Rep.* **2018**, *8*, 4479. [[CrossRef](#)]
41. Vesterbacka, J.; Rivera, J.; Noyan, K.; Parera, M.; Neogi, U.; Calle, M.; Paredes, R.; Sönnnerborg, A.; Noguera-Julian, M.; Nowak, P. Richer gut microbiota with distinct metabolic profile in HIV infected elite controllers. *Sci. Rep.* **2017**, *7*, 6269. [[CrossRef](#)] [[PubMed](#)]
42. Meng, J.; Tao, J.; Abu, Y.; Sussman, D.A.; Girotra, M.; Franceschi, D.; Roy, S. HIV-positive patients on antiretroviral therapy have an altered mucosal intestinal but not oral microbiome. *Microbiol. Spectr.* **2023**, *11*, e0247222. [[CrossRef](#)] [[PubMed](#)]
43. Fontana, A.; Manchia, M.; Panebianco, C.; Paribello, P.; Arzedi, C.; Cossu, E.; Garzilli, M.; Montis, M.A.; Mura, A.; Pisanu, C.; et al. Exploring the role of gut microbiota in major depressive disorder and in treatment resistance to antidepressants. *Biomedicines* **2020**, *8*, 311. [[CrossRef](#)]
44. Manchia, M.; Fontana, A.; Panebianco, C.; Paribello, P.; Arzedi, C.; Cossu, E.; Garzilli, M.; Montis, M.A.; Mura, A.; Pisanu, C.; et al. Involvement of gut microbiota in schizophrenia and treatment resistance to antipsychotics. *Biomedicines* **2021**, *9*, 875. [[CrossRef](#)] [[PubMed](#)]
45. Jiang, H.; Ling, Z.; Zhang, Y.; Mao, H.; Ma, Z.; Yin, Y.; Wang, W.; Tang, W.; Tan, Z.; Shi, J.; et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* **2015**, *48*, 186–194. [[CrossRef](#)]
46. Zheng, P.; Zeng, B.; Zhou, C.; Liu, M.; Fang, Z.; Xu, X.; Zeng, L.; Chen, J.; Fan, S.; Du, X.; et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* **2016**, *21*, 786–796. [[CrossRef](#)] [[PubMed](#)]
47. Wong, M.L.; Inerra, A.; Lewis, M.D.; Mastronardi, C.A.; Leong, L.; Choo, J.; Kentish, S.; Xie, P.; Morrison, M.; Wesselingh, S.L.; et al. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol. Psychiatry* **2016**, *21*, 797–805. [[CrossRef](#)]
48. Inerra, A.; Rogers, G.B.; Licinio, J.; Wong, M.L. The microbiota-inflammasome hypothesis of major depression. *BioEssays* **2018**, *40*, e1800027. [[CrossRef](#)]
49. Barandouzi, Z.A.; Starkweather, A.R.; Henderson, W.A.; Gyamfi, A.; Cong, X.S. Altered composition of gut microbiota in depression: A systematic review. *Front. Psychiatry* **2020**, *11*, 541. [[CrossRef](#)]
50. Jianguo, L.; Xueyang, J.; Cui, W.; Changxin, W.; Xuemei, Q. Altered gut metabolome contributes to depression-like behaviors in rats exposed to chronic unpredictable mild stress. *Transl. Psychiatry* **2019**, *9*, 40. [[CrossRef](#)]

51. Galkin, F.; Mamoshina, P.; Aliper, A.; Putin, E.; Moskalev, V.; Gladyshev, V.N.; Zhavoronkov, A. Human gut microbiome aging clock based on taxonomic profiling and deep learning. *iScience* **2020**, *23*, 101199. [[CrossRef](#)] [[PubMed](#)]
52. Trottein, F.; Sokol, H. Potential causes and consequences of gastrointestinal disorders during a SARS-CoV-2 infection. *Cell Rep.* **2020**, *32*, 107915. [[CrossRef](#)] [[PubMed](#)]
53. Martino, C.; Kellman, B.P.; Sandoval, D.R.; Clausen, T.M.; Marotz, C.A.; Song, S.J.; Wandro, S.; Zaramela, L.S.; Salido Benítez, R.A.; Zhu, Q.; et al. Bacterial modification of the host glycosaminoglycan heparan sulfate modulates SARS-CoV-2 infectivity. *bioRxiv* **2020**. [[CrossRef](#)]
54. Chen, Y.R.; Zheng, H.M.; Zhang, G.X.; Chen, F.L.; Chen, L.D.; Yang, Z.C. High *Oscillospira* abundance indicates constipation and low BMI in the Guangdong Gut Microbiome Project. *Sci. Rep.* **2020**, *10*, 9364. [[CrossRef](#)]
55. Del Chierico, F.; Manco, M.; Gardini, S.; Guarrasi, V.; Russo, A.; Bianchi, M.; Tortosa, V.; Quagliariello, A.; Shashaj, B.; Fintini, D.; et al. Fecal microbiota signatures of insulin resistance, inflammation, and metabolic syndrome in youth with obesity: A pilot study. *Acta Diabetol.* **2021**, *58*, 1009–1022. [[CrossRef](#)]
56. Wang, Z.; Usyk, M.; Sollecito, C.C.; Qiu, Y.; Williams-Nguyen, J.; Hua, S.; Gradissimo, A.; Wang, T.; Xue, X.; Kurland, I.J.; et al. Altered gut microbiota and host metabolite profiles in women with human immunodeficiency virus. *Clin. Infect. Dis.* **2020**, *71*, 2345–2353. [[CrossRef](#)] [[PubMed](#)]
57. Piggott, D.A.; Tuddenham, S. The gut microbiome and frailty. *Transl. Res.* **2020**, *221*, 23–43. [[CrossRef](#)]
58. Haran, J.P.; Bucci, V.; Dutta, P.; Ward, D.; McCormick, B. The nursing home elder microbiome stability and associations with age, frailty, nutrition and physical location. *J. Med. Microbiol.* **2018**, *67*, 40–51. [[CrossRef](#)] [[PubMed](#)]
59. Jackson, M.A.; Jeffery, I.B.; Beaumont, M.; Bell, J.T.; Clark, A.G.; Ley, R.E.; O'Toole, P.W.; Spector, T.D.; Steves, C.J. Signatures of early frailty in the gut microbiota. *Genome Med.* **2016**, *8*, 8. [[CrossRef](#)] [[PubMed](#)]
60. Dickson, I. Gut microbiota: Frailty associated with gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **2016**, *13*, 122.
61. Maffei, V.J.; Kim, S.; Blanchard, E.; Luo, M.; Jazwinski, S.M.; Taylor, C.M.; Welsh, D.A. Biological aging and the human gut microbiota. *J. Gerontol. A Biol. Sci. Med. Sci.* **2017**, *72*, 1474–1482. [[CrossRef](#)] [[PubMed](#)]
62. Li, S.X.; Armstrong, A.; Neff, C.P.; Shaffer, M.; Lozupone, C.A.; Palmer, B.E. Complexities of gut microbiome dysbiosis in the context of HIV infection and antiretroviral therapy. *Clin. Pharmacol. Ther.* **2016**, *99*, 600–611. [[CrossRef](#)] [[PubMed](#)]
63. Campbell, C.; Kandalgaonkar, M.R.; Golonka, R.M.; Yeoh, B.S.; Vijay-Kumar, M.; Saha, P. Crosstalk between gut microbiota and host immunity: Impact on inflammation and immunotherapy. *Biomedicines* **2023**, *11*, 294. [[CrossRef](#)] [[PubMed](#)]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.