



**HAL**  
open science

## **Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen**

Gregory Dubourg, Jean-Christophe J.-C. Lagier, Sophie Hue, Mathieu Surénaud, Dipankar Bachar, Catherine Robert, Caroline Michelle, Isabelle Ravaux, Saadia Mokhtari, Matthieu Million, et al.

### ► To cite this version:

Gregory Dubourg, Jean-Christophe J.-C. Lagier, Sophie Hue, Mathieu Surénaud, Dipankar Bachar, et al.. Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen. *BMJ Open Gastroenterology*, 2016, 3 (1), 10.1136/bmjgast-2016-000080 . hal-01787250

**HAL Id: hal-01787250**

**<https://amu.hal.science/hal-01787250>**

Submitted on 7 May 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

# Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen

Grégory Dubourg,<sup>1,2</sup> Jean-Christophe Lagier,<sup>1</sup> Sophie Hüe,<sup>3,4,5,6</sup> Mathieu Sureau,<sup>3,4,5</sup> Dipankar Bachar,<sup>1</sup> Catherine Robert,<sup>1</sup> Caroline Michelle,<sup>1</sup> Isabelle Ravaut,<sup>7</sup> Saadia Mokhtari,<sup>8</sup> Matthieu Million,<sup>1,8</sup> Andreas Stein<sup>7</sup> Philippe Brouqui,<sup>1,8</sup> Yves Levy,<sup>3,4,5,6,9</sup> Didier Raoult,<sup>1,2,10</sup>

**To cite:** Dubourg G, Lagier J-C, Hüe S, *et al.* Gut microbiota associated with HIV infection is significantly enriched in bacteria tolerant to oxygen. *BMJ Open Gastro* 2016;**3**:e000080. doi:10.1136/bmjgast-2016-000080

► Additional material is available. To view please visit the journal (<http://dx.doi.org/10.1136/bmjgast-2016-000080>)

Received 21 January 2016  
Revised 31 May 2016  
Accepted 3 June 2016

For numbered affiliations see end of article.

**Correspondence to** Professor Didier Raoult; didier.raoult@gmail.com

## ABSTRACT

**Objectives:** Gut microbiota modifications occurring during HIV infection have recently been associated with inflammation and microbial translocation. However, discrepancies between studies justified a comprehensive analysis performed on a large sample size.

**Design and methods:** In a case–control study, next-generation sequencing of the 16S rRNA gene was applied to the faecal microbiota of 31 HIV-infected patients, of whom 18 were treated with antiretroviral treatment (ART), compared with 27 healthy controls. 21 sera samples from HIV-infected patients and 7 sera samples from control participants were used to test the presence of 25 markers of inflammation and/or immune activation.

**Results:** Diversity was significantly reduced in HIV individuals when compared with controls and was not restored in the ART group. The relative abundance of several members of Ruminococcaceae such as *Faecalibacterium prausnitzii* was critically less abundant in the HIV-infected group and inversely correlated with inflammation/immune activation markers. Members of Enterobacteriaceae and Enterococcaceae were found to be enriched and positively correlated with these markers. There were significantly more aerotolerant species enriched in HIV samples (42/52 species, 80.8%) when compared with the control group (14/87 species, 16.1%;  $\chi^2$  test,  $p < 10^{-5}$ , conditional maximum-likelihood estimate (CMLE) OR=21.9).

**Conclusions:** Imbalance between aerobic and anaerobic flora observed in HIV faecal microbiota could be a consequence of the gut impairment classically observed in HIV infection via the production of oxygen. Overgrowth of proinflammatory aerobic species during HIV infection raises the question of antioxidant supplementation, such as vitamin C, E or *N*-acetylcysteine.

## INTRODUCTION

Studies concerning human gut microbiota are continuously growing,<sup>1</sup> and numerous

## Summary box

### What is already known about this subject?

- Gut microbiota disruptions occurring during HIV infection are associated with inflammation, immune activation or microbial translocation.
- Enterobacteriaceae and *Prevotella* spp. are frequently found to be enriched in HIV participants, whereas a decrease in *Bacteroides* spp. is commonly reported.
- Bacterial diversity of HIV gut microbiota is still debated, as well as the impact of antiretroviral treatment (ART) on microbial community restoration.

### What are the new findings?

- Diversity was significantly reduced in HIV individuals and was not restored by ART.
- We found significantly more aerotolerant species enriched in HIV samples when compared with the control group.
- Species intolerant to oxygen such as *Faecalibacterium prausnitzii* or members of the *Ruminococcus* genus were depleted.
- Bacteria positively associated with inflammation were tolerant to oxygen, whereas those negatively associated were strictly anaerobic.

### How might it impact on clinical practice in the foreseeable future?

- Markers of oxidative stress and gut redox potential could help to assess disease progression.
- Imbalance between aerobic and anaerobic communities in HIV participants raises the question of antioxidant supplementation, such as vitamin C, E or *N*-acetylcysteine.

associations with human health and disease have been suggested, including inflammatory bowel diseases, energy metabolism or susceptibility to *Clostridium difficile* infections.<sup>2–9</sup> The number of publications concerning HIV and gut microbiota was previously fairly modest but has also begun to increase in recent years. Indeed, although

there is strong evidence of extensive structural changes in the relative abundance of microorganisms in the HIV faecal bacterial community, significant differences in regard to the sampled material or the platforms used for taxonomic assignment lead to heterogeneous conclusions. This has been particularly reflected in discrepancies concerning gut microbiota diversity during HIV infection, which were increased in some studies<sup>10</sup> and depleted in others,<sup>11–12</sup> while stomach fluid flora diversity has been described as reduced.<sup>13</sup> Alongside these modifications, gut microbiota from US participants infected with HIV-1 have also been described as *Prevotella*-rich or *Bacteroides*-poor, similar to HIV-negative individuals in agrarian societies and in participants with high carbohydrate, low protein and low fat foods,<sup>10</sup> suggesting an interplay with diet. Increase in the *Prevotella* community has nevertheless been linked with increased numbers of activated colonic T cells and myeloid dendritic cells (mDC).<sup>14</sup> Moreover, focusing on colonic mucosal-adherent bacterial microbiota, depletion of Bacteroidales and an increase in Proteobacteria has been reported in HIV rectal biopsies.<sup>15</sup> Interestingly, these disruptions have been associated with acceleration of tryptophan degradation through the kynurenine pathway.<sup>15</sup> This has been positively correlated with gut impairment markers as well as levels of inflammatory cytokines.<sup>16</sup> In addition, proinflammatory states and disease progression in HIV patients have been linked to gut bacterial community disruptions during HIV infection. For example, the high production of interleukin (IL)-6 and increased tumour necrosis factor (TNF)/IL-10 ratio have been associated with increased Enterobacteriaceae<sup>15–17</sup> and *Eubacterium bifforme*,<sup>18</sup> respectively, and TCD8+ cell activation was mediated by Bacteroidales.<sup>19</sup> While antiretroviral treatment (ART) appears to be able to reduce some abnormalities,<sup>12</sup> it alone cannot consistently restore the gut microbiota of HIV participants to their native state.<sup>10–16–18</sup> This is supported by the increase in genes involved in lipopolysaccharide (LPS) biosynthesis, or inflammatory pathways revealed by functional gene content sequencing of gut mucosa from patients receiving ART.<sup>20</sup> Gut microbiota disturbances occurring during HIV infection may alter the functional role of the epithelial barrier towards pathogenic microorganisms.<sup>21–23</sup> Finally, depletion of anaerobic prokaryotes was recently associated with microbiota of severe acute malnutrition,<sup>24</sup> which shares several clinical similarities with the wasting syndrome, a progressive weight loss associated with AIDS, which has not disappeared despite the advent of ART.<sup>25</sup>

Accordingly, this study aimed to assess bacterial diversity and identify a taxonomic signature of HIV gut microbiota, including balance between aerobic species and those intolerant to oxygen. Secondarily, potential associations between enriched or depleted taxa with disease progression markers (CD4 T-cell count, viral load), inflammation markers and translocation markers were tested.

## MATERIALS AND METHODS

### Patients

In a case–control study following the STROBE statement,<sup>26</sup> we compared the gut microbiota of 32 HIV-positive patients to that of 27 non-HIV healthy participants. HIV patients were recruited within the Infectious Diseases Departments in Conception and North Hospitals, Marseille, France, between 2012 and 2014. Non-HIV healthy controls were recruited during the same period and city using a snowball approach. Patients were eligible if they were adults (>18 years), HIV seropositive and provided a stool sample. Data collected included age, sex, antibiotic treatment within the three previous months, weight and height. In addition, we collected for cases only: CD4+ T cells, viral load and ART (see online supplementary table S1). Exclusion criteria included antibiotic treatment within the three previous months and body mass index <18.5 or  $\geq 25$  kg/m<sup>2</sup>. Signed written consent forms were obtained for all participants. Permission from the local ethics committee of the IFR48 (Marseille, France) was obtained under agreement 09-022 and ANRS EP55 MICROGUT.

### Variables

Measured variables included gut bacterial relative abundances at the family, genus and species levels, presence of ART, CD4 T-cell count, viral load and seric concentrations of inflammation and translocation markers. HIV serology was determined with the Architect assay (Abbott Diagnostics, Mannheim, Germany). Plasma HIV RNA testing was performed using the RealTime HIV-1 assay (Abbott Diagnostics).

### DNA extraction and sequencing

All participants' stool samples were stored at  $-80^{\circ}\text{C}$  just after sample collection. Fifty-nine samples were extracted using a deglycosylation protocol, as follows: 250  $\mu\text{L}$  of each sample was placed in a 2 mL tube containing a mixture of acid-washed glass beads (Sigma, Aldrich) and with two or three 0.5 mm glass beads. Mechanical lysis was performed by bead beating the mixture using a FastPrep BIO 101 apparatus (Qbiogene, Strasbourg, France) at maximum speed (6.5) for 3 $\times$ 30 s. The supernatant was centrifuged at 12 000 rpm for 10 min and the pellet retained. A mixture containing 2  $\mu\text{L}$  of 10 $\times$ glycoprotein denaturing buffer EndoHf (New England Biolabs) and 17  $\mu\text{L}$  of H<sub>2</sub>O was added and heated at 100 $^{\circ}\text{C}$  for 10 min. Deglycosylation was performed adding a mixture of 2  $\mu\text{L}$  of 10 $\times$ G5 reaction buffer (ref B1702 New England Biolabs), 2  $\mu\text{L}$  of EndoHf (New England Biolabs), 2  $\mu\text{L}$  of cellulase (Sigma) and 16  $\mu\text{L}$  of H<sub>2</sub>O. The preparation was then incubated overnight at 37 $^{\circ}\text{C}$ . Finally, DNA was extracted using the EZ1 biorobot (Qiagen) with the EZ1 DNA tissues kit. The elution volume was 50  $\mu\text{L}$ .

Samples were amplified individually for the 16S 'V3\_V4' regions by PCR on MiSeq technology using the Taq Phusion (Thermo Fisher Scientific, Waltham,

Massachusetts, USA) and the surrounding conserved regions V3\_V4 primers with overhang adapters (FwOvAd\_341F CGTCCGGCAGCGTCAGATGTGTATAAG AGACAGCCTACGGGNGGCWGCAG; RevOvAd\_785RG TCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACT ACHVGGGTATCTAATCC). After purification on AMPure beads (Beckman Coulter, Fullerton, California, USA), concentrations were measured using high sensitivity Qubit technology (Beckman Coulter) and dilutions to 0.2 ng/μL were performed. Using a subsequent limited cycle PCR on 1 ng of each PCR product, Illumina sequencing adapters and dual-index barcodes were added to each amplicon. After purification on AMPure beads (Beckman Coulter), libraries were then normalised according to the Nextera XT protocol (Illumina, San Diego, California, USA). The multiplexed samples were pooled into a single library for sequencing on the MiSeq (Illumina). Automated cluster generation and paired-end sequencing with dual index reads was performed in a single 39 hour run in a 2×250 bp. These samples were mixed with other amplicon projects, resulting in four runs being conducted. On average, a total of 7.86 Gb of information was obtained from a 1030 K/mm<sup>2</sup> cluster density, with a cluster passing 74.48% quality control filters (21 587 600 clusters). Within these runs, the index representations for all samples were determined between 0.38% and 3.5%. Raw data were configured in fasta files for R1 and R2 reads as paired-end reads.

The paired-end reads from the Illumina Miseq raw fasta files were assembled into contigs using FLASH (Fast Length Adjustment of SHort reads),<sup>27</sup> a tool to merge paired-end reads from next-generation sequencing experiments when the original DNA fragments are shorter than twice the length of reads. FLASH was used to clean the sequences at the same time of merging paired-end reads with a (OFFSET) quality score cut-off of 33. The longer joined sequences obtained from FLASH were filtered using QIIME,<sup>28</sup> trimming off the primers and removing the sequences shorter than 200 nts and >1000 nts. The chimeric sequences were also removed using the QIIME chimerafilter. Clustering of sequences into operational taxonomic units (OTUs) was performed by UCLUST<sup>29</sup> and the QIIME de novo method at 97% similarity, without considering the singletons.

### Building the reference database

The SILVA SSU and LSU database<sup>30</sup> (release 119) was downloaded from the SILVA website and used to build a local database of predicted amplicon sequences. While building our local reference database, we only extracted SILVA SSU reference sequences if and only if they contained both the forward and reverse primers, allowing three differences between each primer and the SILVA reference sequences. Finally, our local reference database contained a total of 456 714 well-annotated sequences. One sample from an HIV participant was

finally excluded from the study due to insufficient meta-genomic data (<40 000 total high-quality filtered reads).

### Taxonomic assignment

The OTUs (representative sequences) extracted in the previous step were blasted<sup>31</sup> against our local reference database. The 100 best matches above 80% identity (100% coverage and e value of 0.000001) with each of the representative sequences were extracted from the reference database.

These extracted reference sequences with the SILVA taxonomy were sorted according to the decreasing percentage of similarity. The reference sequences with the highest percentage of similarity (also considering all the hits within a 0.5% similarity of the best hits) with the OTUs were then considered for taxonomic assignment. Taxonomy to the lowest rank was obtained by applying a majority voting.<sup>32</sup> When similarity was 80%, sequences were not assigned. Finally, all the tags were clustered to different taxon ranks according to the consensus taxonomy of the unique tags (representative of each OTU).<sup>33–35</sup> Rarefaction was not performed as it could lead to false-positive results.<sup>36</sup> The details of sequence numbers and OTUs can be seen in online supplementary table S3. All the sequences in raw fastq format have been submitted to EMBL-EBI<sup>37</sup> with the accession number PRJEB10578.

### Disease progression markers and systemic inflammation profiles

Twenty-one sera samples from HIV-infected patients of which 15 under ART and 7 sera samples from non-HIV-infected participants were collected to test the presence of 25 markers of inflammation and/or immune activation. sCD14 and FABP-2/I-FABP were quantified using ELISA (Bio-Techne, Abingdon, UK). The other markers were quantified using Luminex multiplex bead-based technology and a Bio-Plex 200 instrument (Bio-Rad): IL-2Rα, IL-18, MIG/CXCL9 and TRAIL using Bio-plex Pro Human cytokine Group II (Bio-Rad, Marnes-La-Coquette, France); B7-H1/PD-L1, MIP-1α/CCL4, sCD27, sCD30, sCD40L, sCD163, CXCL16, interferon (IFN)-γ, IL-17A, IL-22, IL-18BPα, IL-6, IL-8/CXCL8, IL-10, IP-10/CXCL10, I-TAC/CXCL11, MCP-1/CCL2, TNF-α and MIP-3α/CCL20 using Human Magnetic Luminex screening Assay (Bio-Techne, Abingdon, UK) in line with the manufacturer's instructions. One replicate was performed for each assay.

### Statistical analyses

Shannon diversity indices (H') were calculated for each sample using the formula  $H' = -\sum p_i \times \ln(p_i)$ .<sup>38</sup> A subgroup analysis was planned a priori differentiating patients with and without ART. Statistical analyses were performed using GraphPad Prism, V.5.0 (La Jolla, California, USA). The Fisher's exact test was used for dichotomous variables, the Mann-Whitney test for continuous variables and the Spearman test for correlations.

Principal coordinate analysis (PCoA) was obtained using the weighted unifracs distance after data rarefaction at the depth of 50 000 reads per sample, and the Adonis test was performed in QIIME. Linear discriminant analysis effect size (LEfSe) was performed on relative abundances at the genus and species levels using parameters recommended by Segata *et al.*,<sup>39</sup> including per-sample normalisation of the sum of the values to 1 M (<http://huttenhower.sph.harvard.edu/galaxy/>). Tolerance to oxygen for each species or genus was checked using the 'List of Prokaryotes according to their Aerotolerant or Obligate Anaerobic Metabolism' (<http://www.mediterranee-infection.com/article.php?laref=374&titre=list-of-prokaryotes-according-to-their-aerotolerant-or-obligate-anaerobic-metabolism>). Owing to the possible risk of missing important findings, adjustments for multiple comparisons were not performed, as suggested for exploratory work.<sup>40</sup> However, in a sensitivity analysis to test the robustness of our results, we re-analysed our data set based on the relative abundance of each species after normalisation to 50 000 reads (samples with <50 000 16S rRNA reads were excluded, and all relative abundance inferior to 1/50 000 were converted to 0). A non-parametric Kruskal-Wallis test was thus used with an adjustment for multiple comparison using the post hoc Benjamini-Hochberg correction from the OMICS package in XLSTAT V.2016.02 (Addinsoft, Paris, France).

## RESULTS

### Participants

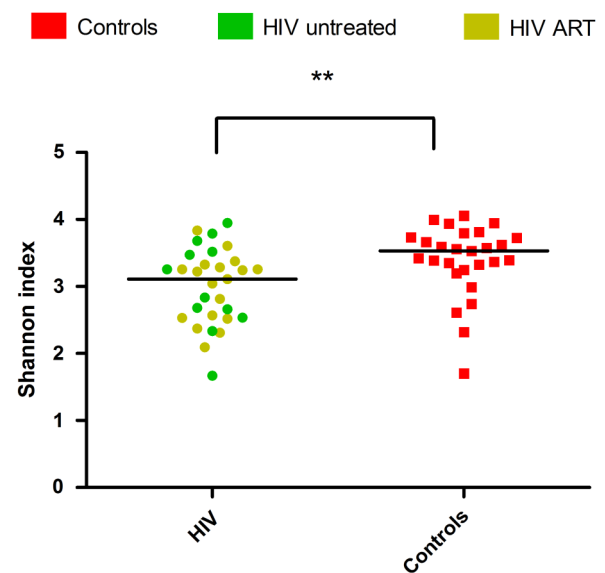
All variables had been assessed for the 59 participants (32 HIV and 27 healthy controls). However, one seropositive individual had been excluded because of the insufficient number of reads (<40 000; see online supplementary table S2).

### Decreased diversity of gut microbiota from HIV participants

The  $\alpha$ -diversity was significantly reduced in HIV individuals when compared with controls as shown by the lower Shannon index ( $H' = 2931$  and  $3394$ , respectively;  $p = 0.0068$ ). Diversity was not restored in the ART group when compared with the untreated group ( $H' = 2974$  and  $2879$ , respectively;  $p = 0.72$ ; [figure 1](#)). However, data clustering analysed by PCoA using the weighted unifracs distance to obtain the PCoA in the QIIME pipeline shows marked separation between controls and HIV individuals (ADONIS test  $p = 0.002$ ), as between HIV-treated and HIV-untreated participants (ADONIS test,  $p = 0.033$ ; see online supplementary figure S1).

### Taxa discriminating HIV-negative and seropositive participants

All HIV individuals clearly show a significant decrease in the Clostridia class as compared with the control samples ( $p < 0.0001$ ; [figure 2](#)). Peptostreptococcaceae



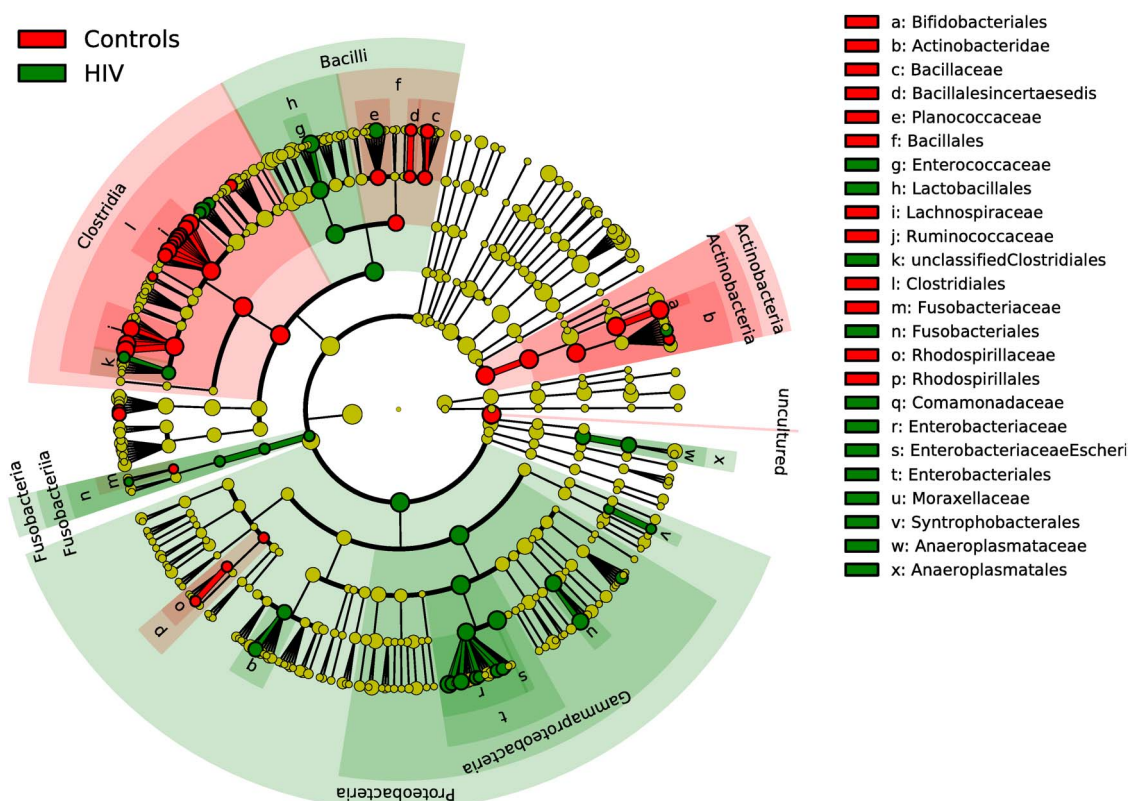
**Figure 1** Shannon indices applied on HIV cases and non-HIV controls. ART, antiretroviral treatment.

families ( $p = 0.0216$ ) and especially Ruminococcaceae ( $p < 0.0001$ ) were significantly decreased. More specifically, the genera *Subdoligranulum* ( $p = 0.0002$ ), *Ruminococcus* ( $p < 0.0001$ ), *Blautia* ( $p = 0.0239$ ) and *Faecalibacterium* ( $p = 0.0354$ ) were critically less abundant. At the species level, *R. bromii* ( $p < 0.0001$ ), *R. obeum* ( $p = 0.0086$ ), *F. prausnitzii* ( $p < 0.0001$ ), *B. luti* ( $p = 0.0199$ ) and *B. faecis* ( $p = 0.0385$ ) were decreased.

The Gammaproteobacteria class was significantly increased ( $p = 0.0021$ ) in HIV-infected participants ([figure 2](#)), including the *Citrobacter* genera ( $p = 0.03$ ) and *Escherichia coli* ( $p = 0.0368$ ).

Moreover, we observed a low relative abundance of *Bifidobacterium* genera ( $p = 0.0458$ ) and *B. adolescentis* ( $p = 0.0111$ ) at the species level in HIV-infected individuals, while the *Enterococcus* genus ( $p = 0.0339$ ), including *E. faecalis* ( $p = 0.0248$ ) and *E. faecium* ( $p = 0.0376$ ), were found to be enriched.

We also performed LEfSe at the species level to strengthen our results. LEfSe indeed confirmed enrichment of species belonging to Proteobacteria as diminution those belonging to Clostridia in HIV individuals ([figure 2](#)). Unprofitable taxa were removed and genera and species enriched in each group with an Linear Discriminant Analysis (LDA) score  $> 2.0$  were then kept. Interestingly, there were significantly more aerotolerant genera in the HIV group (12/18, 66.7%) than in the control group (5/29, 17.2%;  $\chi^2$  test,  $p = 4.8 \times 10^{-4}$ , CMLE OR=9.1; see online supplementary figure S2). Similarly, there were significantly more aerotolerant species enriched in HIV samples (42/52 species, 80.8%) when compared with the control group (14/87 species, 16.1%;  $\chi^2$  test,  $p < 10^{-5}$ , conditional maximum-likelihood estimate (CMLE) OR=21.9; [figure 3](#)). Even though LDA is a validated method,<sup>38</sup> we tested the robustness of our



**Figure 2** Cladogram yielding taxa enriched in each case and control group with an LDA score >2.

results performing an adjustment for multiple comparison (Benjamini-Hochberg, see the Materials and methods section) and normalisation to 50 000 reads. These secondary analyses confirmed our main result with a very significant depletion of anaerobic bacteria in HIV gut microbiota (CMLE OR=11.1,  $p=5.5 \times 10^{-5}$ ; see online supplementary table S4). We then compared sequences from HIV individuals included in this study with those of uninfected controls from work by Dinh *et al*<sup>11</sup> (project accession number SRP039076). Taxonomic assignment and LEfSe at the genus level were performed as described in the Materials and methods section. There were significantly more enriched aerotolerant genera in HIV included here (40/63, 63.5%) than in controls from the previously cited study (8/63, 12.7%;  $\chi^2$  test,  $p < 10^{-5}$ , CMLE OR=11.7; see online supplementary figure S3).

### Disease progression markers and systemic inflammation profiles

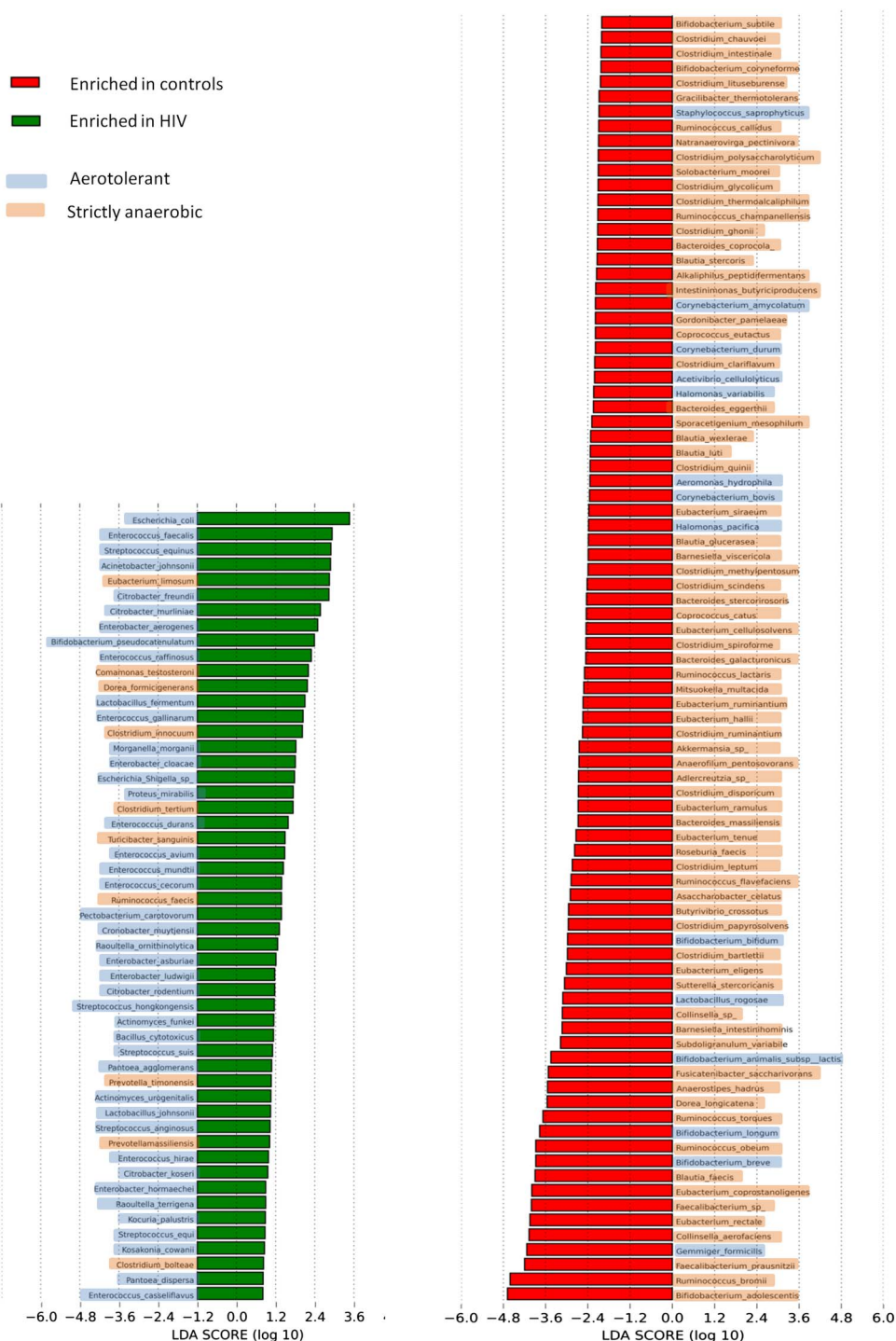
Of the 25 markers studied, 7 (B7-H1/PD-L1, IFN-g, IL-17A, IL-22, IL-6, IL-10 and TNF- $\alpha$ ) were below the lower limit of quantification and 12 were shown to be significantly different between HIV+ patients and HIV- participants using the Mann-Whitney rank test (see online supplementary figure S4). Chemokines and T-cell activation markers are correlated (see online supplementary figure S5), whereas no correlation was found between T-cell activation markers and translocation markers. These results suggested different mechanisms

involved in the activation of T cells and monocytes/macrophages. We then looked at Spearman correlations between the relative abundance of bacteria families or species and levels of these biomarkers (table 1). Members of the *Ruminococcaceae* family (especially *R. bromii* and *F. prausnitzii*) were inversely correlated with inflammation/immune activation markers when depleted. Members of the *Enterobacteriaceae* family (especially *E. coli* and *Enterobacter aerogenes*) and of the *Enterococcaceae* family (*E. faecalis* and *E. faecium*) were positively correlated with these markers when enriched (table 1). Altogether, these results highlight that dysbiosis observed in HIV-infected patients is associated with systemic immune activation.

### DISCUSSION

In this paper, we propose a metagenomic approach to stool samples collected from 31 HIV individuals and 27 controls. Identical special procedures and protocols were used from DNA extraction to amplicon analysis to ensure the robustness of our results.

First, our results found reduced gut microbiota diversity during HIV infection, revealed by lower Shannon indices in the HIV group independently of ART (figure 1). This point remains currently controversial with publications either confirming<sup>11</sup> or contradicting this statement.<sup>10</sup> A reduction in the bacterial diversity has recently been reported in the stomach fluid microflora of HIV-positive patients<sup>13</sup> and this could be



**Figure 3** LDA scores of differentially abundant species among cases and controls and their tolerance to oxygen according to the 'List of Prokaryotes according to their Aerotolerant or Obligate Anaerobic Metabolism'. The LDA scores represent the effect size of each abundant species. Species enriched in each group with an LDA score  $>2$  are considered.

linked to bowel diseases.<sup>42</sup> Alongside this modification, high structural changes had been observed in the HIV group, with a switch between species belonging to the Clostridia and those belonging to the Gammaproteobacteria classes, which were found to be, respectively, decreased and increased. Deep taxonomic analysis revealed a dramatic decrease in *R. bromii*, a

keystone member of Ruminococcaceae for starch hydrolysis,<sup>43</sup> in HIV patients. Interestingly, activation and maturation status of colonic mDC in HIV patients had been negatively associated with a low prevalence of mucosal species, including *R. bromii*.<sup>44</sup> In addition, downregulation of starch metabolism had been observed in the intestinal epithelial barrier occurring during

**Table 1** Significant associations found between different analysed markers (translocation markers, T-cell activation markers and chemokines) and specific taxa

Taxon	Depleted or enriched in HIV	Marker	Marker type	R-Spearman	p Value
<i>Blautia faecis</i>	Depleted	IP-10/CXCL10	Chemokine	-05 047	00 062
		sCD27	T-cell activation	-04 712	00 131
		sCD30	T-cell activation	-04 131	00 289
<i>Enterobacter aerogenes</i>	ND	I-FABP	Translocation	03 992	00 353
		IP-10/CXCL10	Chemokine	0434	0021
		sCD27	T-cell activation	03 821	00 492
Enterococcaceae	Enriched	MIG/CXCL9	Chemokine	04 209	00 257
		sCD163	Translocation	05 348	0004
		sCD27	T-cell activation	03 936	00 422
<i>Enterococcus faecalis</i>	Enriched	IP-10/CXCL10	Chemokine	04 149	00 281
		MIG/CXCL9	Chemokine	0433	00 214
		sCD163	Translocation	04 022	00 375
		sCD27	T-cell activation	04 785	00 116
<i>Faecalibacterium prausnitzii</i>	Depleted	IP-10/CXCL10	Chemokine	-06 201	00 004
		MIG/CXCL9	Chemokine	-04 505	00 161
		MIP-3a/CCL20	Chemokine	-03 759	00 487
		sCD14	Translocation	-04 618	00 153
		sCD27	T-cell activation	-03 939	00 421
		sCD30	T-cell activation	-04 822	00 094
<i>Ruminococcus bromii</i>	Depleted	IP-10/CXCL10	Chemokine	-04 302	00 223
		MIG/CXCL9	Chemokine	-03 916	00 393
		sCD163	Translocation	-04 858	00 102
<i>R. faecis</i>	Depleted	IP-10/CXCL10	Chemokine	05 803	00 062
		MIG/CXCL9	Chemokine	-04 597	00 138
		MIP-3A/CCL20	Chemokine	-04 209	00 257
		sCD14	Translocation	-04 815	0011
		sCD27	T-cell activation	-06 367	00 004
		sCD30	T-cell activation	-04 476	00 169
<i>R. torques</i>	Depleted	IP-10/CXCL10	Chemokine	-04 419	00 185
		MIP-3a/CCL20	Chemokine	-05 177	00 048
		sCD14	Translocation	-04 872	0,01
		sCD27	T-cell activation	-04 311	00 248

primary HIV infection.<sup>45</sup> Besides, we found *F. prausnitzii* to be less abundant in HIV patients, which had been strongly associated with inflammatory bowel diseases such as Crohn's disease or colitis<sup>46–48</sup> as the result of its anti-inflammatory properties which lead to its potential use as a probiotic.<sup>49</sup> An increase in Gammaproteobacteria resulted from the high Enterobacteriaceae count, including the genera *Citrobacter* and *Escherichia-Shigella* and, more specifically, *E. coli*. Our findings are supported by a recent publication<sup>41</sup> showing enrichment in Enterobacteriaceae, which are well known to be immune system activators and proinflammatory organisms through LPS mediation.<sup>17</sup> Similar changes have also been observed in rectal biopsy microbiota from HIV participants.<sup>15</sup> These disruptions are evidence of a deep imbalance between aerobic and anaerobic flora, which was still found when amplicon sequences from healthy participants<sup>41</sup> from another study were considered as controls. The increase in oxygen within the gastrointestinal tract could be a consequence of the gut impairment classically observed in HIV infection, promoting facultative anaerobes. Interestingly,

the increased bacteria associated with elevated activation immune markers were aerotolerant (table 1). In the same way, microorganisms for which depletion was correlated to the same activation immune markers were strictly anaerobic (5/5; table 1), and mainly concern the Ruminococcaceae family, in particular *F. prausnitzii* and *R. bromii* (table 1). To the best of our knowledge, this is the first report on the aerobic/anaerobic bacteria imbalance in HIV gut microbiota. However, it is the overall dysbiosis, possibly due to intestinal barrier impairment, which appears to be the cause of this proinflammatory state, rather than one particular species. These findings raise the question of the involvement of gut microbiota in oxidative stress occurring during HIV infection, as well as the possible introduction of vitamin C supplementation, which has been previously suggested to have a protective effect.<sup>50 51</sup> In addition, in vitro studies demonstrated that supplementation with vitamin C and *N*-acetylcysteine in combination reduced early apoptosis of CD4+ T-cell activation induced by LPS.<sup>52</sup> Moreover, selenium supplementation protects infected T cells against an H<sub>2</sub>O<sub>2</sub> cytotoxic



effect, increasing glutathione peroxidase and decreasing nuclear factor- $\kappa$ B activation.<sup>53</sup> Unfortunately, the impact of antioxidants on gut microbiota composition has not been studied, to the best of our knowledge, even if their use in microbiology can promote growth of anaerobes in the presence of oxygen.<sup>54</sup>

We assume that the low number of concomitant sera collected (N=27) and the fact that diet had not been taken into account constitute limitations of this work. In addition, it would be interesting to sample mucosal specimens rather than faeces samples.

In conclusion, this study highlights the loss of gut microbiota diversity during HIV infection, associated with an increase in aerobic bacteria such as Enterobacteriaceae or Enterococcaceae, and the depletion of anaerobic bacteria, mainly represented by the Ruminococcaceae family. The association of these disruptions with several activation or translocation markers raises the question of gut bacterial community restoration as an adjuvant treatment for HIV infection.

#### Author affiliations

<sup>1</sup>Faculté de Médecine, URMITE, UMR CNRS 6236-IRD 198, Aix-Marseille Université, Marseille, France

<sup>2</sup>Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bactériologie-Hygiène-Virologie, University Hospital Centre Timone, Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, Assistance Publique—Hôpitaux de Marseille, Marseille, France

<sup>3</sup>INSERM, U955, Equipe 16, Créteil, 94000, France

<sup>4</sup>Université Paris Est, Faculté de médecine, Créteil, France

<sup>5</sup>Vaccine Research Institute (VRI), Créteil, France

<sup>6</sup>AP-HP, Hôpital H. Mondor—A. Chenevier, Service d'immunologie biologique, Créteil, France

<sup>7</sup>Service de Maladies Infectieuses et tropicales, CHU de la Conception, 147, boulevard Baille, Pôle Infectieux, Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France

<sup>8</sup>Assistance Publique Hôpitaux de Marseille, CHU Nord, Pôle Infectieux, Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France

<sup>9</sup>AP-HP, Hôpital H. Mondor—A. Chenevier, Service d'immunologie clinique et maladies infectieuses, Créteil, France

<sup>10</sup>Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

**Acknowledgements** The authors wish to thank Emile Foucat for his valuable help in assaying the markers analysed in this study.

**Contributors** DR designed the whole study, monitored data collection, as well as drafted and revised the paper. GD monitored the data collection, analysed the data, as well as drafted and revised the paper. J-CL initiated the collaborative project, as well as drafted and revised the paper. PB, AS, SM and IR monitored the sample collection and revised the draft paper. CR and CM monitored the metagenomics analysis, as well as drafted and revised the paper. DB analysed the metagenomics data, as well as drafted and revised the paper. MS, SH and YL performed the correlation markers analysis, as well as drafted and revised the paper. MM performed the statistical analysis and revised the paper.

**Funding** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Ethics approval** Approval from local ethics committee of the IFR48 (Marseille, France) was obtained under agreement 09-022.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** No additional data are available.

**Open Access** This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

#### REFERENCES

- Lagier JC, Million M, Hugon P, *et al*. Human gut microbiota: repertoire and variations. *Front Cell Infect Microbiol* 2012;2:136.
- Chang JY, Antonopoulos DA, Kalra A, *et al*. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis* 2008;197:435–8.
- Frank DN, St Amand AL, Feldman RA, *et al*. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007;104:13780–5.
- Sokol H, Seksik P, Rigottier-Gois L, *et al*. Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm Bowel Dis* 2006;12:106–11.
- Tumbaugh PJ, Ley RE, Mahowald MA, *et al*. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–131.
- Zhang H, DiBaise JK, Zuccolo A, *et al*. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci USA* 2009;106:2365–70.
- Larsen N, Vogensen FK, van den Berg FW, *et al*. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE* 2010;5:e9085.
- Qin J, Li Y, Cai Z, *et al*. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55–60.
- Tong M, McHardy I, Ruegger P, *et al*. Reprogramming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism. *ISME J* 2014;8:2193–206.
- Lozupone CA, Li M, Campbell TB, *et al*. Alterations in the gut microbiota associated with HIV-1 infection. *Cell Host Microbe* 2013;14:329–39.
- Mutlu EA, Keshavarzian A, Losurdo J, *et al*. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. *PLoS Pathog* 2014;10:e1003829.
- McHardy IH, Li X, Tong M, *et al*. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. *Microbiome* 2013;1:26.
- von Rosenfinge EC, Song Y, White JR, *et al*. Immune status, antibiotic medication and pH are associated with changes in the stomach fluid microbiota. *ISME J* 2013;7:1354–66.
- Dillon SM, Lee EJ, Kotter CV, *et al*. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunol* 2014;7:983–94.
- Vujkovic-Cvijin I, Dunham RM, Iwai S, *et al*. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med* 2013;5(193):193ra91.
- Jenabian MA, El-Far M, Vyboh K, *et al*. Montreal Primary Infection and Slow Progressor Study Groups. Immunosuppressive tryptophan catabolism and gut mucosal dysfunction following early HIV infection. *J Infect Dis* 2015;212:355–66.
- Marchetti G, Bellistri GM, Borghi E, *et al*. Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy. *AIDS* 2008;22:2035–8.
- Lozupone CA, Rhodes ME, Neff CP, *et al*. HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. *Gut Microbes* 2014;5:562–70.
- Ellis CL, Ma ZM, Mann SK, *et al*. Molecular characterization of stool microbiota in HIV-infected subjects by panbacterial and order-level 16S ribosomal DNA (rDNA) quantification and correlations with immune activation. *J Acquir Immune Defic Syndr* 2011;57:363.
- Vázquez-Castellanos JF, Serrano-Villar S, Latorre A, *et al*. Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. *Mucosal Immunol* 2015;8:760–72.
- Ferreira RB, Gill N, Willing BP, *et al*. The intestinal microbiota plays a role in Salmonella-induced colitis independent of pathogen colonization. *PLoS ONE* 2011;6:e20338.

22. Gori A, Tincati C, Rizzardini G, *et al.* Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. *J Clin Microbiol* 2008;46:757–8.
23. Sharma R, Young C, Neu J. Molecular modulation of intestinal epithelial barrier: contribution of microbiota. *J Biomed Biotechnol* 2010;2010:305879.
24. Million M, Tidjani Alou M, Khelaifia S, *et al.* Increased gut redox and depletion of anaerobic and methanogenic prokaryotes in severe acute malnutrition. *Sci Rep* 2016;6:26051. (in press).
25. Mangili A, Murman DH, Zampini AM, *et al.* Nutrition and HIV infection: review of weight loss and wasting in the era of highly active antiretroviral therapy from the nutrition for healthy living cohort. *Clin Infect Dis* 2006;42:836–42.
26. von Elm E, Altman DG, Egger M, *et al.* STROBE Initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ* 2007;335:806–8.
27. Magoç T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 2011;27:2957–63.
28. Caporaso JG, Kuczynski J, Stombaugh J, *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;7:335–6.
29. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;26:2460–1.
30. Quast C, Pruesse E, Yilmaz P, *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;41:D590–6.
31. Altschul SF, Gish W, Miller W, *et al.* Basic local alignment search tool. *J Mol Biol* 1990;215:403–10.
32. Guillou L, Bachar D, Audic S, *et al.* The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Res* 2013;41:D597–604.
33. Boissière A, Tchioffo MT, Bachar D, *et al.* Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. *PLoS Pathog* 2012;8:e1002742.
34. Stoeck T, Behnke A, Christen R. Massively parallel tag sequencing reveals the complexity of anaerobic marine protistan communities. *BMC Biol* 2009;7:72.
35. Terrat S, Christen R, Dequiedt S, *et al.* Molecular biomass and MetaTaxogenomic assessment of soil microbial communities as influenced by soil DNA extraction procedure. *Microb Biotechnol* 2012;5:135–41.
36. McMurdie PJ, Holmes S. Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Comput Biol* 2014;10:e1003531.
37. Squizzato S, Park YM, Buso N, *et al.* The EBI Search engine: providing search and retrieval functionality for biological data from EMBL-EBI. *Nucleic Acids Res* 2015;43:W585–8.
38. Hutcheson K. A test for comparing diversities based on the Shannon formula. *J Theor Biol* 1970;29:151–4.
39. Segata N, Izard J, Waldron L, *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.
40. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43–6.
41. Dinh DM, Volpe GE, Duffalo C, *et al.* Intestinal microbiota, microbial translocation, and systemic inflammation in chronic HIV infection. *J Infect Dis* 2015;211:19–27.
42. Ott SJ, Musfeldt M, Wenderoth DF, *et al.* Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004;53:685–93.
43. Ze X, Duncan SH, Louis P, *et al.* *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. *ISME J* 2012;6:1535–43.
44. Dillon SM, Lee EJ, Kotter CV, *et al.* Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T-cell activation in untreated HIV-1 infection. *Mucosal Immunol* 2016;9:24–37.
45. Sankaran S, George MD, Reay E, *et al.* Rapid onset of intestinal epithelial barrier dysfunction in primary human immunodeficiency virus infection is driven by an imbalance between immune response and mucosal repair and regeneration. *J Virol* 2008;82:538–45.
46. Jia W, Whitehead RN, Griffiths L, *et al.* Is the abundance of *Faecalibacterium prausnitzii* relevant to Crohn's disease? *FEMS Microbiol Lett* 2010;310:138–44.
47. Sokol H, Seksik P, Furet JP, *et al.* Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis* 2009;15:1183–9.
48. Sokol H, Pigneur B, Watterlot L, *et al.* *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008;105:16731–6.
49. Van Immerseel F, Ducatelle R, De Vos M, *et al.* Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. *J Med Microbiol* 2010;59:141–3.
50. Stephensen CB, Marquis GS, Jacob RA, *et al.* Vitamins C and E in adolescents and young adults with HIV infection. *Am J Clin Nutr* 2006;83:870–9.
51. Allard JP, Aghdassi E, Chau J, *et al.* Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS* 1998;12:1653–9.
52. Mburu S, Marnewick JL, Abayomi A, *et al.* Modulation of LPS-induced CD4+ T-cell activation and apoptosis by antioxidants in untreated asymptomatic HIV infected participants: an in vitro study. *Clin Dev Immunol* 2013;2013:631063.
53. Sappéy C, Legrand-Poels S, Best-Belpomme M, *et al.* Stimulation of glutathione peroxidase activity decreases HIV type 1 activation after oxidative stress. *AIDS Res Hum Retroviruses* 1994;10:1451–61.
54. Dione N, Khelaifia S, La Scola B, *et al.* A quasi-universal medium to break the aerobic/anaerobic bacterial culture dichotomy in clinical microbiology. *Clin Microbiol Infect* 2016;22:53–8.