Journal of Microbiology, Immunology and Infection 56 (2023) 311—323

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Microbiota analysis in the hemodialysis population - Focusing on *Enterobacteriaceae*

Hon-Yen Wu [a,b,c,d,1], Yi-Tsung Lin [e,f,1], Wan-Chuan Tsai [a,g], Yen-Ling Chiu [a,h,i], Mei-Ju Ko [j,k], Ju-Yeh Yang [a], Mei-Fen Pai [a], Hsin-Sui Hsu [a], Shih-Ping Hsu [a,l], Yu-Sen Peng [a,m,n], Chun-Hsing Liao [a,b,*]

[a] *Department of Internal Medicine, Far Eastern Memorial Hospital, New Taipei City, Taiwan*
[b] *School of Medicine, College of Medicine, National Yang Ming Chiao Tung University, Taipei City, Taiwan*
[c] *Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei City, Taiwan*
[d] *Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei City, Taiwan*
[e] *Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, Taipei City, Taiwan*
[f] *Institute of Emergency and Critical Care Medicine, National Yang Ming Chiao Tung University, Taipei City, Taiwan*
[g] *Center for General Education, Lee-Ming Institute of Technology, New Taipei City, Taiwan*
[h] *Graduate Program in Biomedical Informatics, Yuan Ze University, Taoyuan City, Taiwan*
[i] *Graduate Institute of Medicine, Yuan Ze University, Taoyuan City, Taiwan*
[j] *Department of Dermatology, Taipei City Hospital, Taipei City, Taiwan*
[k] *Department of Dermatology, National Taiwan University Hospital and College of Medicine, Taipei City, Taiwan*
[l] *School of Life Science, National Taiwan Normal University, Taipei City, Taiwan*
[m] *Department of Applied Cosmetology, Lee-Ming Institute of Technology, New Taipei City, Taiwan*
[n] *Department of Healthcare Administration, Asia Eastern University of Science and Technology, New Taipei City, Taiwan*

Received 25 July 2022; received in revised form 8 November 2022; accepted 4 December 2022
Available online 9 December 2022

* Corresponding author: Division of Infectious Disease, Department of Internal Medicine, Far Eastern Memorial Hospital. No. 21, Sec. 2, Nanya S. Rd., Banciao Dist., New Taipei City 220, Taiwan.
E-mail address: liaoch@femh.org.tw (C.-H. Liao).
[1] Dr. Hon-Yen Wu and Dr. Yi-Tsung Lin contributed equally as first authors of this work.

https://doi.org/10.1016/j.jmii.2022.12.001

KEYWORDS
Enterobacteriaceae;
Gastrointestinal microbiome;
Hemodialysis;
Kidney failure;
Chronic;
Microbiota;
Prognosis

Abstract *Background:* Infection is a recognized risk factor for mortality among hemodialysis (HD) population, including infection caused by *Enterobacteriaceae*. We aimed to investigate *Enterobacteriaceae* in gut microbiota among HD patients and to analyze associations between microbiota and clinical parameters.
Methods: This prospective study of microbiota analysis in HD patients was conducted in April—May 2019. A control group without recent antibiotic use or hospitalization was used for comparison. Stool samples underwent 16S rRNA sequencing, using Greengenes 16S rRNA database for microbiota analysis.
Results: Among 96 hemodialysis (HD) patients, mean age was 61.9 ± 0.8 years and mean duration of HD was 6.5 ± 0.7 years. No significant differences were found in alpha diversity between HD and control groups (HD group 949.5, controls 898; $p = 0.16$) although significant between-group differences were found in beta diversity ($p < 0.001$). At phylum level, HD group had a higher abundance of *Firmicutes* and *Proteobacteria*, but lower abundance of *Bacteriodetes*. At genus level, *Escherichia-Shigella* complex increased among HD patients who had hospitalization with 1 year (median 0.024 vs 0.004, $p = 0.054$) and *Klebsiella* was associated with emergency room visit within 1 year among HD patients ($p = 0.002$).
Conclusions: Alpha diversity in HD patients is not lower than that in healthy controls but significant between-group differences are found in microbiota composition according to beta diversity, due to decreased *Bacteriodetes* and increased *Firmicutes* and *Proteobacteria*. Deeper microbiota analyses for *Enterobacteriaceae* are necessary. Whether change in dietary components can help to decrease mortality among dialysis population warrants further research.

Introduction

The gut microbiota is considered to have an important role in human disease, and microbiota-mediated therapy has been proposed accordingly.[1,2] However, many factors affect the composition of the microbiota, including food, lifestyle and medications.[3,4] Associations between microbiota and other diseases have been under vigorous research, but the effects were less prominent and limited due to the complexity of the investigation.[1,2,5,6] Patients receiving hemodialysis (HD) regularly are a special population. They have regular hospital exposure, multiple medications, specific dietary considerations and frequent medical events.[7–10] Only limited reports have been published about microbiota analysis among HD patients.[11–14] The study of Vaziri et al. showed that microbiota from patients with end-stage renal disease (ESRD) was markedly different from that of healthy controls,[11] and in rat uremic models the difference was mostly due to decreases in operational taxonomic units (OTUs) in the *Lactobacillaceae* and *Prevotellaceae* families. In a pediatric study of ESRD, the relative abundance of *Bacteroidetes* (phylum) was increased in HD patients ($p = 0.046$), and the relative abundance of *Proteobacteria* ($p = 0.023$) was increased in peritoneal dialysis patients.[13] On the contrary, a review of the gut microbiome in patients with chronic kidney disease (CKD), found a lower abundance of *Bifidobacteriaceae* and *Lactobacillaceae* and higher levels of *Enterobacteriaceae*.[14]

Bacterial infection is a common complication among HD patients, and bacteremia caused by *Staphylococcus* from the skin is well-documented among HD cases.[8,15,16] However, bacteremia caused by *Enterobacteriaceae* is less studied.[15,17–19] Evidence of detection of circulating bacterial-derived DNA fragments has been found among ESRD patients (around 20%) and was linked to inflammation markers.[20,21] Among the fragments, DNA from *Enterobacteriaceae* predominates. With the advancement of 16S rRNA sequencing technology and analysis,[22,23] stool microbiota analysis has become a more feasible tool to understand the role of microbiota for a specific condition. This prospective study aimed to investigate the potential role of *Enterobacteriaceae* in gut microbiota among hemodialysis patients who did not have sepsis at the time of sample collection, and to analyze associations between the microbiota and clinical parameters.

Methods

Study design, setting, and sample

This prospective study enrolled HD patients from the dialysis center of the Far Eastern Memorial Hospital (FEMH), a 1400-bed tertiary hospital in northern Taiwan. The HD center serves about 380 cases regularly in an out-patient setting. The National Health Insurance in Taiwan universally covers patients' expenses for HD.[24,25] The initial enrollment was evaluated by nephrologists. Patients who had difficulty collecting stool samples (e.g., poor eyesight, bed-ridden status, etc.) were excluded. Those who were willing to cooperate were then enrolled. A structured case record form, including underlying illness, duration of HD, body mass index (BMI), medications, recent infection/hospitalization, and labora-

tory parameters was used for all included patients. Dietary protein intake was assessed by the normalized protein catabolic rate (nPCR).[26] Charlson comorbidity index (CCI) scores were calculated based on patients' comorbidities.[27] All stool samples were collected and stored in the fridge at the patients' homes prior to HD sessions if the stool could not be processed by the study team within 3 h. Samples were stored at the −80 °C freezer until tested. All patients were followed for more than one year after enrollment. Healthy control cases were also collected since 2017 at FEMH (age >20 years, no major systemic disease, no antibiotics within 3 months).

16S rRNA gene sequencing

Total genomic DNA was directly extracted using QIAmp Fast DNA Stool Mini kit (Qiagen, Germany) according to the manual for product instruction from frozen samples. After isolation, DNA yield was approximately 50ug and the DNA concentration is 150ng/ul. The DNA sample was stored at −20 °C before polymerase chain reaction (PCR) amplification. For the PCR amplification step, the forward and reverse primers that were complementary upstream and downstream of the V3–V4 region of 16S were designed with Illumina overhang adapters and employed to amplify templates from bacterial genomic DNA. PCR products were purified with a GeneHlow Gel/PCR purification kit (Geneaid, Taiwan). For the Index PCR and clean up a step, Illumina sequencing adapters and dual indices were attached to the PCR products using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). Subsequently, AMPure XP beads were used to clean up the final libraries, and the expected size on a Bioanalyzer trace of the final libraries was approximately 630 bp. For the normalization and sequencing steps, Libraries were normalized and pooled and sequenced on the MiSeq system using v3.0 reagents (pair-end 250bp, Illumina, San Diego, CA, USA).

Sequence quality control

The following four major steps were used to analyze the sequence reads in FASTAQ format: (a) Check the quality value of raw reads using FastQC[28]; (b) Merge pair-end reads of a sample according to the overlap sequences using the PEAR software,[29] and discard the overlapped sequence less than 10 bp; (c) Clusters of similar sequences with at least 97% identified were defined as an OTU using UCLUST.[30] Then, the QIIME software package (version 1.9.1)[31] with default setting was applied to compare sequences to a reference database using the Greengenes database (release 13_8).[32]

Statistical analyses

The alpha diversity, beta diversity, microbiome structure, multi-variant statistical analysis, and co-occurrence network analysis plots are generated using R software (R Foundation for Statistical Computing, Vienna, Austria). Comparison between HD group and healthy control was performed according to the abundance of genus level among HD patients and with linear discriminant analysis effect size (LefSe). For the analysis between specific OTUs and clinical parameters, correlation coefficient were calculated and Mann-Whitney U test and Pearson's chi-square test were used for p values. Data were analyzed with SPSS software version 15.0 for Windows (SPSS, Chicago, IL, USA).

Results

Characteristics of HD patients/healthy controls and overall diversity of gut microbiota

During the study period (April–May 2019), 96 HD patients were enrolled, including 66 men and 30 women. Mean age was 61.9 ± 0.8 years and mean duration of hemodialysis was 6.5 ± 0.7 years. The average BMI was 23.8 ± 0.4 kg/m^2, and median CCI score was 6 (standard deviation ± 2). The healthy control group ($n = 81$) was younger (44.4 ± 1.4 years old, $p < 0.05$) than the HD group. Moreover, the healthy control cohort contained 62 women and only 19 men ($p < 0.05$), but the BMI was similar 23.8 ± 0.4 kg/m^2 ($p = 0.93$). Comparison between the HD and control groups showed that the median observed species was 949.5 for the HD group and 898 for the control group ($p = 0.16$) (Fig. 1A). However, significant differences were found in beta diversity between the two groups (Fig. 1B (unweighted) and 1C (weighted); $p < 0.001$). BMI was associated with observed species in the HD group (correlation coefficient (r) = 0.31; $p = 0.002$), but the association was not found in healthy controls ($r = 0.08$; $p = 0.50$). Two HD patients died within one-year follow-up. (alpha diversity analyzed with chao1, faith's phylogenetic, and diversity Shannon diversity offered as supplementary data).

Microbiota comparison at phylum level

Fig. 2 shows the differences in microbiota between the HD and control groups at the phylum level. The HD group had a higher abundance of *Firmicutes* (median 0.58 [0.1–0.96] vs. 0.53 [0.09–0.88]; $p = 0.034$) and *Proteobacteria* (median 0.06 [0–0.68] vs. 0.02 [0–0.68]; $p = 0.005$), but a lower abundance of *Bacteriodetes* (median 0.12 [0.002–0.63] vs. 0.29 [0.002–0.82]; $p = 0.001$). The HD group also had a higher abundance of *Fusobacteria* than the controls but the abundance was still very low in both groups (median 0 [0.002–0.33] vs. 0 [0–0.49]; $p = 0.01$).

The result of LEfSe and microbiota comparison at the genus level

The result of LEfSe was shown in Fig. 3. In brief, abundance of *Escherichia*, *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Staphylococcus*, *Klebsiella* increased among HD cases, while *Bifidobacterium*, *Prevotella*, and *Bacteroides* decreased. Table 1 shows results of further analysis of the distribution of major OTU identities (IDs) at the genus level based on abundance in the HD group. The most common OTU ID among *Firmicutes* was *Blautia* (ID 532203), which

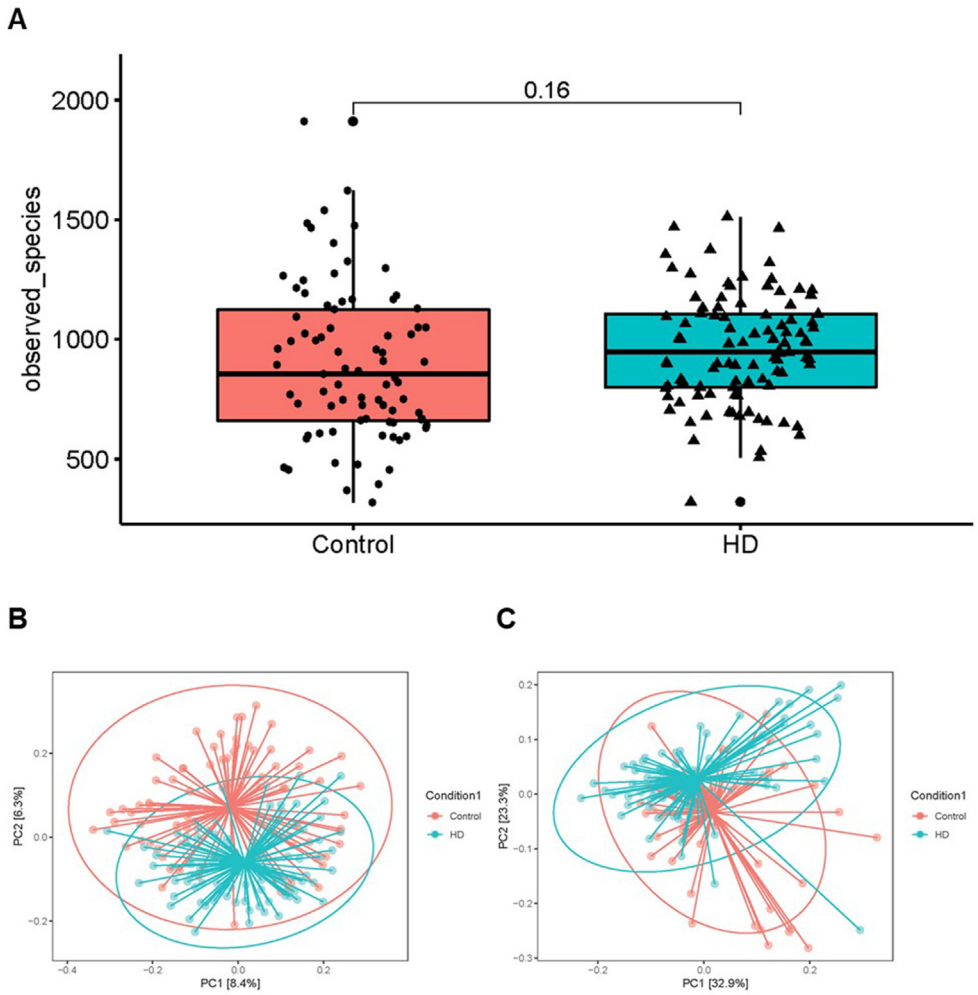


Fig. 1. Comparison between hemodialysis (n = 96) and healthy control (n = 81) groups by (A) alpha diversity (p = 0.16), (B) unweighted beta diversity (p = 0.0001), and (C) weighted beta diversity (p = 0.0001). HD, hemodialysis.

was significantly higher than that in the healthy controls group (median 0.012 vs. 0.005; p = 0.017). The most common OTU among *Proteobacteria* was *Escherichia-Shigella* complex (ID 1111294), which was significantly higher compared to that in the controls (median 0.009 vs. 0.002; p < 0.001). In contrast, the most common OTU ID among *Bacteriodetes* was *Bacteroides* (ID 589277), which was significantly lower than that in the healthy controls group (median 0.009 vs. 0.018; p = 0.036).

Given the significant increase of *Proteobacteria* among HD cases, the top 20 abundant OTUs are listed in Table 2. All OTU IDs increased in the HD group. The top five IDs were identified as *Escherichia-Shigella* complex (3), *Escherichia* and *Klebsiella*. The distribution of OTUs among healthy controls was similar, but the relative abundance was lower. Five HD cases had *Proteobacteria* abundance higher than 50%, and details of relative abundance in these 5 cases are shown in Fig. 4. OTU 1111294 is most prevalent among these cases, ranging from 39.4% to 23.5%, followed by OTU 114510 (8.5%–10.1%). The abundance of OTU 813127 (genus *Klebsiella*) varied from 0% to 12.5%.

The abundance of genus *Blautia* in the HD group is unexpected (OTU 532203, OTU 366846, OTU 183684). All three OTUs were positively associated with alpha diversity (r = 0.37, p < 0.001, r = 0.36, p < 0.001, and r = 0.36, p < 0.001) and negatively associated with phylum *Proteobacteria* (r = −0.24, p = 0.017, r = −0.27, p = 0.008, r = −0.22, p = 0.03). No significant correlations were found between genus *Blautia* and BMI (p > 0.1 for all 3 OTUs) or genus *Bifidobacterium* (p > 0.41 for all three OTUs). These 3 OTUs were strongly associated with alpha diversity (r = 0.54, p < 0.001, r = 0.62, p < 0.001, r = 0.54, p < 0.001) and genus *Bifidobacterium* (r = −0.39, p < 0.001, r = 0.46, p < 0.001, r = 0.40, p < 0.001) in healthy controls and negatively associated with phylum *Proteobacteria* (r = −0.48, p < 0.001; r = −0.48, p < 0.001; r = −0.47, p < 0.001). The median relative abundance of genus *Bifidobacterium* was 0.004 among HD group patients (range 0–0.25) and 0.007 among healthy controls (range 0–0.44), p = 0.03.

Clostridium difficile (OTU 606927) was of special interest and therefore analyzed. The median relative abundance of OTU 606927 was 3.35E-05 among the HD group (range 0–0.005) and 0 among healthy controls (range 0–0.005); p < 0.001. Analysis of genus *Clostridium* (contains more than 10 OTUs) revealed that the median relative abundance

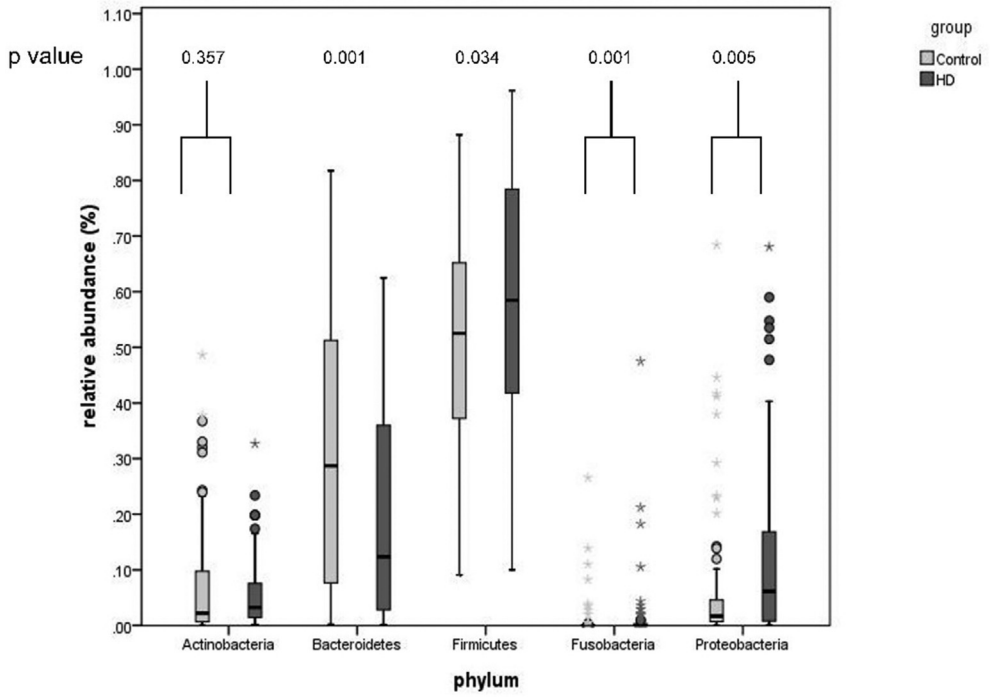


Fig. 2. Comparison between hemodialysis and healthy control groups at the phylum level. HD, hemodialysis.

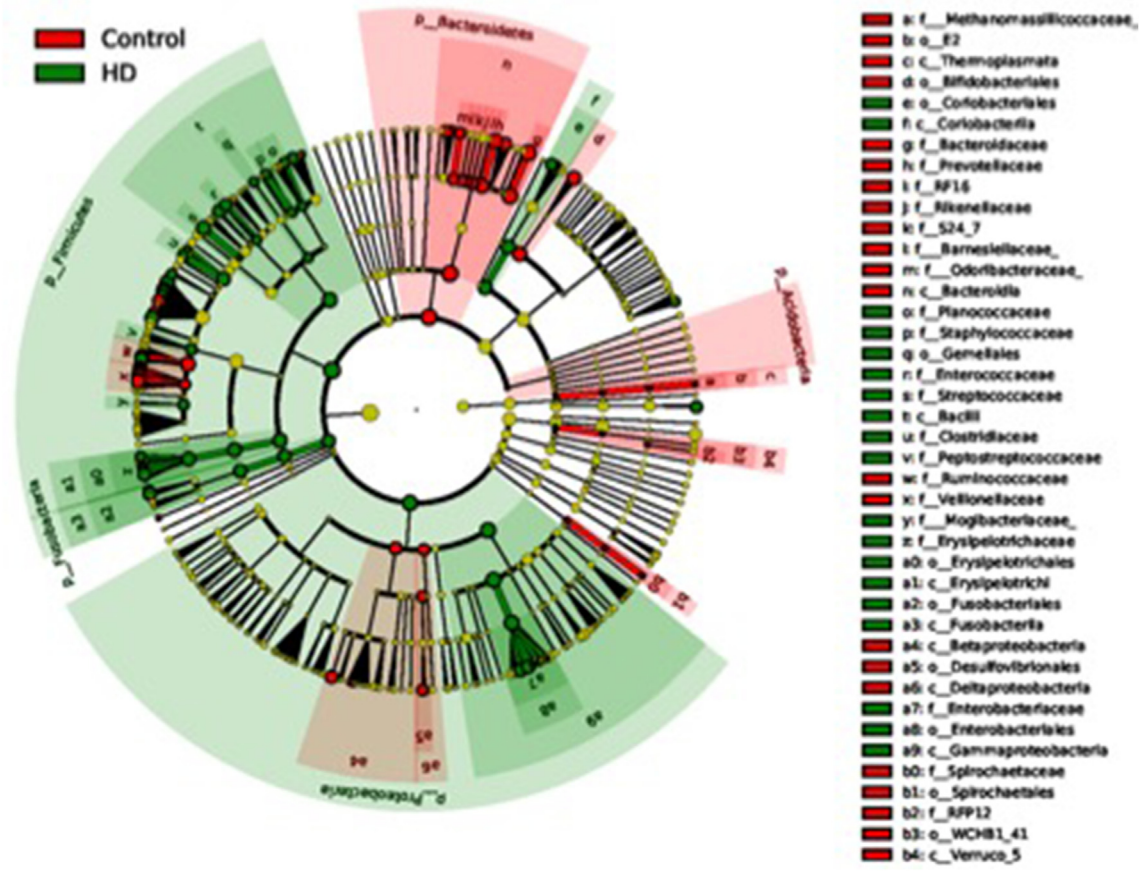


Fig. 3. LEfSe comparison between hemodialysis and healthy control at the genus level.

of *Clostridium* was 0.0007 among the HD group (range 0–0.23) and 0.0008 among healthy controls (range 0–0.01); p = 0.17. The median abundance of genus *Staphylococcus* was 0 among the HD group (range 0–0.027) and 0 among healthy controls (range 0–0.001); p = 0.026.

Correlation between clinical parameters and major *Proteobacteria* OTUs

Table 3 shows the correlations between clinical parameters and abundance of major *Proteobacteria* OTUs. At phylum level, no clinical factors correlated significantly with *Proteobacteria*. At genus level, ID 1111294 (*Escherichia-Shigella* complex) increased among HD patients hospitalized within 1 year (median 0.024 vs. 0.004; p = 0.054). ID abundance of 813217 (*Klebsiella*) correlated negatively with BMI (p = 0.039), was higher among females (p = 0.042), and was associated with emergency room visits during the 1-year follow up period (p = 0.002). Four patients died in the 1-year period, but no associations were noted with major OTUs.

Associations between laboratory parameters and major *Proteobacteria* OTUs are shown in Table 4. At the phylum level, abundance of *Proteobacteria* is associated with lower albumin (≥3.5 g/dL 0.057 vs. <3.5 g/dL 0.168; p = 0.045), nPCR (≥1.2 0.045 g/kg/day vs. <1.2 0.088 g/kg/day, p = 0.015), and BUN (<78 mg/dL 0.088 vs. ≥78 mg/dL 0.024; p = 0.003), which may reflect that relatively poor nutritional status is associated with *Proteobacteria*. At the genus level, both abundance of 1111294 and 114510 were associated with higher hemoglobin and lower nPCR, blood urea nitrogen (BUN), ferritin and intact parathyroid hormone (iPTH).

Quality control

Sequencing quality scores. N = 177. Avg. reads = 85.84. Q30 indicates the probability of an incorrect base call 1 in 1000 times, which means that the base call accuracy (i.e., the probability of a correct base call) was 99.9%. For our criteria, at least 75 percent of the reads in each sample were greater than Q30 and would be used for further analysis. Avg. raw reads = 145119. Avg. effective reads = 101597.

Discussion

Marked advancement in intestinal microbiota analysis in recent years has enhanced our knowledge of the complex human microbiome but clinical correlations and applications are still limited. Meanwhile, the HD cohort is an ideal candidate for delineating the potential role of gut microbiota because it is already subject to special dietary guidelines. In the present analysis, HD patients were shown to have a higher abundance of *Proteobacteria* in spite of reserved diversity. Genus ID 813217 (*Klebsiella*) was associated with emergency room visits during 1-year follow up. Genus *Blautia* was the most abundant among our HD cohort. The abundance of *C. difficile* and genus *Staphylococcus* was low in HD patients but higher than that found in healthy controls.

Table 1 Major OTUs abundance in hemodialysis patients at genus level, compared to healthy controls (based on median abundance in hemodialysis group).

OTU ID	Phylum	Class	Order	Family	Genus	Hemodialysis (n = 96)				Healthy controls (n = 81)				Mann-Whitney U	
						Median	IQR	Mean	SE	Median	IQR	Mean	SE	p value	
532203	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	1.22E-02	1.85E-02	1.71E-02	1.93E-03	5.45E-03	1.31E-02	1.05E-02	1.31E-03	0.017[a]	↑
360015	*Firmicutes*	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	1.16E-02	2.91E-02	2.99E-02	5.60E-03	3.25E-04	1.52E-03	2.02E-03	5.17E-04	<0.001[a]	↑
589277	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	9.48E-03	4.31E-02	3.40E-02	5.25E-03	1.78E-02	6.68E-02	4.63E-02	6.90E-03	0.036[a]	↓
1111294	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	9.44E-03	6.14E-02	6.15E-02	1.03E-02	1.52E-03	6.30E-03	1.55E-02	4.78E-03	<0.001[a]	↑
366846	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	6.46E-03	9.04E-03	9.65E-03	1.06E-03	1.12E-03	4.04E-03	3.71E-03	5.79E-04	<0.001[a]	↑
183684	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	6.25E-03	9.77E-03	8.86E-03	9.83E-04	2.69E-03	6.69E-03	5.09E-03	6.40E-04	0.003[a]	↑
470382	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Anaerobutyricum-Anaerostipes*	5.34E-03	2.20E-02	1.29E-02	1.69E-03	2.40E-03	1.26E-02	8.66E-03	1.50E-03	0.127	↑
547913	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Anaerobutyricum-Anaerostipes*	4.96E-03	2.13E-02	1.41E-02	2.04E-03	2.55E-03	6.12E-03	5.77E-03	1.04E-03	0.007[a]	↑
696563	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	4.54E-03	9.78E-03	8.73E-03	1.07E-03	1.91E-03	5.70E-03	3.71E-03	4.73E-04	<0.001[a]	↑
362997	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	3.14E-03	1.23E-02	1.23E-02	2.65E-03	3.48E-03	2.78E-02	2.03E-02	4.39E-03	0.307	↓
583117	*Bacteroidetes*	*Bacteroidia*	*Bacteroidales*	*Bacteroidaceae*	*Bacteroides*	2.92E-03	7.90E-03	9.17E-03	1.83E-03	2.77E-03	8.95E-03	6.56E-03	9.31E-04	0.836	↑
363794	*Actinobacteria*	*Coriobacteriia*	*Coriobacteriales*	*Coriobacteriaceae*	*Collinsella*	2.82E-03	2.29E-02	1.72E-02	3.05E-03	4.76E-03	2.69E-02	1.94E-02	3.04E-03	0.112	↓
579608	*Firmicutes*	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	2.71E-03	1.57E-02	2.42E-02	5.66E-03	1.08E-03	3.03E-03	3.65E-03	1.14E-03	<0.001[a]	↑
357449	*Firmicutes*	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Anaerobutyricum-Eubacterium -Lactobacillus*	2.57E-03	9.05E-03	5.59E-03	7.39E-04	1.08E-03	5.50E-03	3.68E-03	6.56E-04	0.048[a]	↑
114510	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia*	2.47E-03	1.51E-02	1.59E-02	2.61E-03	1.49E-04	7.21E-04	1.35E-03	3.52E-04	<0.001[a]	↑

[a] $p < 0.05$.

Note. IQR, interquartile range; OTU, operational taxonomic unit; SE standard error.

Table 2 Major *Proteobacteria* OTUs abundance in hemodialysis patients at genus level, compared to healthy controls.

OTU ID	Class	Order	Family	Genus	Hemodialysis (n = 96)				Healthy control (n = 81)				Mann-Whitney U p value	
					Median	IQR	Mean	SE	Median	IQR	Mean	SE		
1111294	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	9.44E-03	3.94E-01	6.15E-02	1.03E-02	1.52E-03	6.30E-03	1.55E-02	4.78E-03	<0.001[a]	↑
114510	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia*	2.47E-03	1.01E-01	1.59E-02	2.61E-03	1.49E-04	7.21E-04	1.35E-03	3.52E-04	<0.001[a]	↑
813217	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Klebsiella*	3.25E-04	1.25E-01	1.01E-02	2.30E-03	1.13E-04	1.91E-03	6.71E-03	2.27E-03	0.056	↑
797229	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	1.79E-04	1.21E-02	1.54E-03	2.69E-04	2.16E-05	1.71E-04	4.51E-04	1.78E-04	<0.001[a]	↑
3829957	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia- Shigella*	1.45E-04	1.82E-01	8.51E-03	2.90E-03	2.73E-05	3.05E-04	1.81E-03	6.67E-04	<0.001[a]	↑
2281837	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	1.20E-04	5.09E-03	7.47E-04	1.26E-04	0.00E+00	2.85E-05	4.63E-05	1.17E-05	<0.001[a]	↑
231787	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia- Shigella*	1.16E-04	7.95E-03	1.00E-03	1.73E-04	9.45E-06	6.07E-05	8.26E-05	2.13E-05	<0.001[a]	↓
730437	*Deltaproteobacteria*	*Desulfovibrionales*	*Desulfovibrionaceae*	*Cupidesulfovibrio-Desulfovibrio*	1.06E-04	5.55E-03	5.35E-04	9.44E-05	4.35E-04	1.20E-03	1.09E-03	2.12E-04	0.002[a]	↑
511908	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Klebsiella*	7.83E-05	6.73E-02	4.79E-03	1.12E-03	2.72E-05	7.29E-04	3.36E-03	1.77E-03	0.015[a]	↑
782953	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	6.59E-05	3.78E-03	4.90E-04	8.20E-05	9.15E-06	4.81E-05	1.08E-02	6.47E-03	<0.001[a]	↑
922761	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Pantoea-Erwinia*	6.19E-05	3.65E-02	1.31E-03	4.59E-04	5.46E-05	2.34E-04	8.95E-04	2.96E-04	0.290	↑
166908	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia- Shigella*	5.54E-05	3.25E-03	3.87E-04	7.23E-05	7.91E-06	4.85E-05	1.09E-04	4.11E-05	<0.001[a]	↑
1104638	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia-Shigella*	5.48E-05	2.52E-03	3.36E-04	5.57E-05	0.00E+00	2.05E-05	1.69E-05	4.17E-06	<0.001[a]	↑
588216	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Escherichia- Shigella*	4.01E-05	2.95E-03	3.32E-04	6.09E-05	0.00E+00	2.48E-05	8.39E-05	3.17E-05	<0.001[a]	↑
581021	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacter*	3.27E-05	1.32E-02	4.27E-04	1.58E-04	1.97E-05	9.88E-05	2.84E-03	2.43E-03	0.279	↑

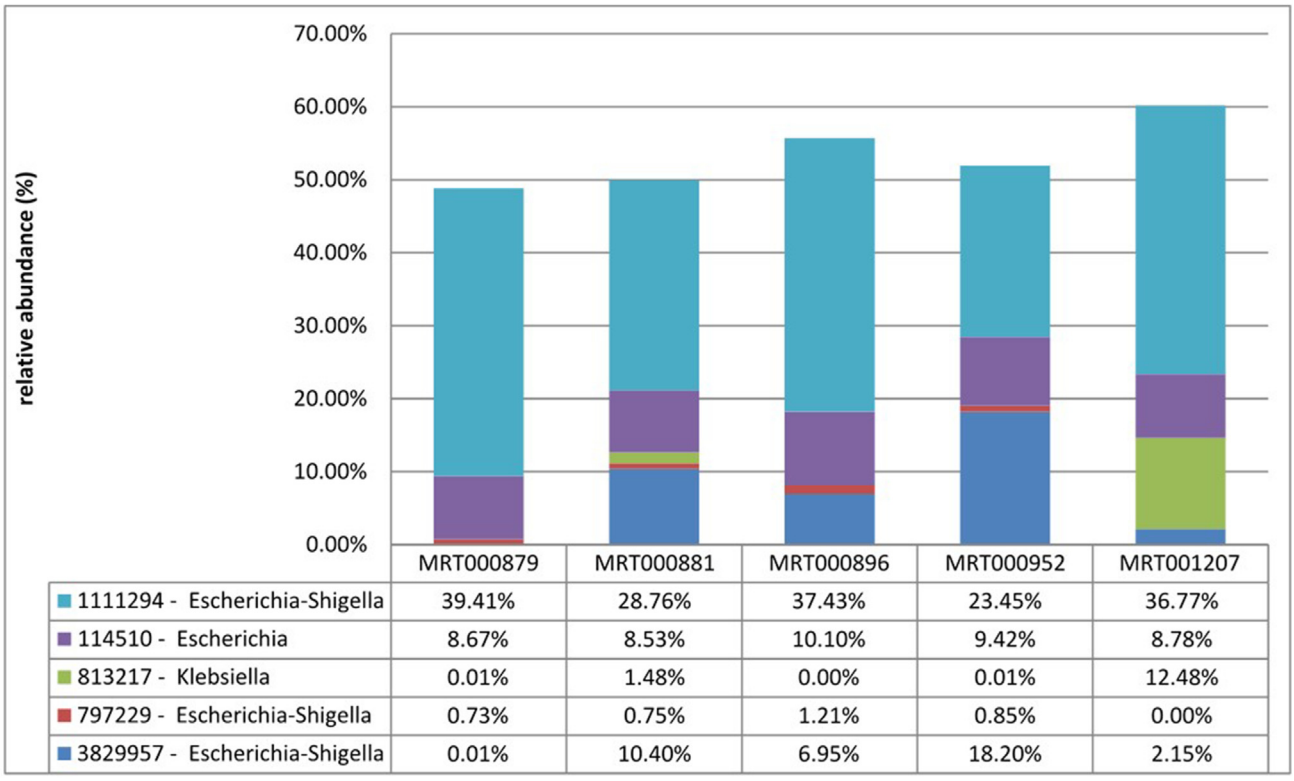
[a] $p < 0.05$.

Note. IQR, interquartile range; OTU, operational taxonomic unit; SE, standard error.

The alpha diversity of our HD cases is not lower than that of healthy controls,[33] but the composition of microbiota is quite different according to beta diversity. Comparison at the phylum level showed that the main difference is due to decreased *Bacteriodetes* and increased *Firmicutes* and *Proteobacteria*, similar to previous reports.[11–14,34] The increased *Proteobacteria* is of special interest since infection/bacteremia caused by *Proteobacteria* is clinically important. Several previous reports/reviews showed elevated abundance of *Escherichia coli* among Crohn's disease patients and *Proteobacteria* among patients with liver cirrhosis.[2,35,36] In a recent study focusing on gut microbiota among CKD patients, 7 genera (*Escherichia-Shigella*, *Dialister*, *Paraprevotella*, and so on) and 2 species (*Collinsellastercoris* and *Bacteriodeseggerthii*) were considered to be CKD-associated microbiota, and levels of *Escherichia-Shigella* spp. correlated positively with disease severity ($r = 0.3$, $p < 0.0001$).[33] Such reports urged us to perform deeper microbiota analysis for *Enterobacteriaceae*.

A longitudinal follow-up study for *Enterobacteriaceae* among healthy adults showed little stability of *Enterobacteriaceae*, demonstrated by a dynamic phylogroup shift.[37] Still, among their 32470 single colonies, 87% belonged to ten *E. coli sensu stricto* and non-*E. coli*, ranging from <1% to 37% between different individuals. In our cross-sectional results among 96 HD cases, *Escherichia-Shigella* complex comprised 10 of the 20 most abundant forms in the phylum *Proteobacteria* and 19 of the top 20 in the genus increased in the HD group. *E. coli* is a major pathogen known for centuries but detailed typing for *E. coli* is complicated and only 20% of the genome is conserved among *E. coli* strains; also, some *E. coli* strains are host-specific and associated with a specific lifestyle.[38] In the present analysis, some HD cases had *Enterobacteriaceae* abundance exceeding 50%, and OTU 1111294 was always the most abundant. We have no information about whether the OTU annotation by 16sRNA V3–V4 sequencing correlated with Clermont phylotyping for *E. coli*.[39] It is possible that full-length 16sRNA (1500 bp) sequencing would have achieved better resolution for strain identification (not just the genus in this study).[40]

Among the top 20 OTUs within the *Proteobacteria* (HD group), 19 were *Enterobacteriaceae*.[41] A recent update in the taxonomy of *Enterobacteriaceae* based on core and ribosomal proteins comprised 21 conserved signature inserts and deletions (CSIs), and *Pantoea-Erwinia* was considered to be another family in the new classification. *Klebsiella* and *Enterobacter* remained in the family of *Enterobacteriaceae* and were found in the top 20. Both of these bacteria were also important pathogens among HD patients. Clinically, detection of the Gram-negative pathogens ranked from *E. coli* and *Klebsiella* to *Enterobacter*, which correlated well with the list. In fact, enteric bacteria from the gut has been considered the source of bacteremia for decades, and domination of *E. coli* and *Enterococcus faecium* in the gut is shown to occur prior to bacteremia.[42] Rectal colonization of *Klebsiella pneumoniae* is associated with higher risk of subsequent infection among high-risk hosts (colonization prevalence 23%, odds ratio 4.06; $p < 0.001$), but the effect is not evident among community diabetes cases.[43,44] Disease severity in the host may be the



	MRT000879	MRT000881	MRT000896	MRT000952	MRT001207
1111294 - Escherichia-Shigella	39.41%	28.76%	37.43%	23.45%	36.77%
114510 - Escherichia	8.67%	8.53%	10.10%	9.42%	8.78%
813217 - Klebsiella	0.01%	1.48%	0.00%	0.01%	12.48%
797229 - Escherichia-Shigella	0.73%	0.75%	1.21%	0.85%	0.00%
3829957 - Escherichia-Shigella	0.01%	10.40%	6.95%	18.20%	2.15%

Fig. 4. Major OTUs among top 5 hemodialysis cases with Proteobacteria abundance. OTU, operational taxonomic unit.

key to subsequent infection. Whether microbiota analysis can predict future infection is of clinical relevance, but only if the turnaround time for analysis can be shortened.

We tried to identify the clinical significance of phylum *Proteobacteria* or specific genus OTUs among HD cases, but only ID 1111294 (*Escherichia-Shigella*) increased among HD patients who were hospitalized within 1 year. Also, ID 813217 (*Klebsiella*) correlated negatively with BMI ($p = 0.039$), was higher among females ($p = 0.042$), and was associated with emergency room visits during the 1-year follow-up period ($p = 0.002$). Two patients died in the 1-year period, but no associations were found with major OTUs. Each HD patient has specific individual conditions and it is unlikely that a single OTU would have a strong predictive power in a single measurement. Previously, *K. pneumoniae* was found to be increased in metabolic liver disease and cardiac disease, while *E. coli* was found to be decreased in cardiac disease.[2] In contrast, a peritoneal dialysis study (using real-time PCR) found that *Bifidobacteria*/*Lactobaciilus*/*K. pneumoniae* were decreased compared to those in healthy controls, and no significant differences were found even in *E. coli* between these groups.[45]

Analysis of laboratory parameters showed that the abundance of *Proteobacteria* was associated with lower levels of albumin, nPCR and BUN, which may reflect relatively poor nutritional status. Undernutrition is associated with increased *Proteobacteria* in early life but overnutrition is also associated with some members of the *Enterobacteriaceae* family.[46] The associations between specific gut microbe and iPTH/hemoglobin/ferritin were less prominent, suggesting that these parameters were probably manipulated by patients' primary care physicians. A trend was noted showing that high-sensitivity C-reactive protein was associated with higher abundance of *Proteobacteria*/*Enterobacteriaceae*, but without statistical significance.

Blautia is of special interest since it is the most abundant genus among the HD cases.[47,48] *Blautia* species are part of butyrate-producing bacteria and are associated with intake of wheat bran extract enriched in arabinoxylans; depletion of *Blautia* species in obese children is associated with intestinal inflammation.[47] Furthermore, *Blautia* was the only gut microbe significantly and inversely associated with visceral fat, which is strongly associated with cardiovascular disease.[48] Among HD patients in the present study, *Blautia* was inversely associated with *Proteobacteria*. We asked further if this result was due to special dietary habits in Taiwan. Animal-based protein is still suggested as a cause, but plant-based protein, mainly soybeans, is also a popular food in Taiwan and is thus a candidate. Plant-based diets are shown to decrease inflammatory markers among HD patients.[49] Only two deaths occurred in one-year follow-up in the present study, compared to 90.1% one-year survival in the incident dialysis patients in Taiwan.[50] This can be explained by our inclusion of only patients who were able to cooperate with stool sample collection. Whether good dietary habits can help to decrease mortality among HD cases warrants further prospective research.

Bifidobacterium is shown to be decreased among HD cases. Food plays a central role for gut microbiota. The Western diet, in particular, is associated with an abundance

Table 3 Correlations between clinical parameters and abundance of major *Proteobacteria* OTUs.

		Proteobacteria (phylum)			1111294 (*Escherichia-Shigella*)		114510 (*Escherichia*)		813217 (*Klebsiella*)	
		n	CC/Median (IQR)	p value	CC/Median (IQR)	p value	CC/Median (IQR)	p value	CC/Median (IQR)	p value
Age		96	0.044	0.67	0.013	0.90	0.019	0.86	−0.013	0.90
Duration of HD		96	0.054	0.61	0.137	0.18	0.137	0.18	0.023	0.82
CCI		96	−0.015	0.88	−0.074	0.47	−0.069	0.50	0.037	0.72
BMI		96	−0.041	0.69	−0.026	0.80	−0.029	0.78	−0.211	0.039[a]
Sex	female	30	0.089 (0.159)	0.16	0.028 (0.073)	0.29	0.007 (0.017)	0.33	0.001 (0.030)	0.042[a]
	male	66	0.044 (0.154)		0.006 (0.015)		0.002 (0.015)		0.000 (0.003)	
Vascular catheter	No	81	0.061 (0.161)	0.62	0.006 (0.060)	0.16	0.002 (0.015)	0.18	0.000 (0.007)	0.40
	Yes	15	0.074 (0.167)		0.032 (0.130)		0.007 (0.032)		0.000 (0.004)	
ABX within 3 months	No	67	0.051 (0.124)	0.22	0.008 (0.061)	0.18	0.002 (0.015)	0.16	0.000 (0.005)	0.69
	Yes	29	0.086 (0.231)		0.024 (0.139)		0.006 (0.037)		0.000 (0.016)	
PPI within 3 months	No	85	0.061 (0.157)	0.25	0.008 (0.060)	0.13	0.002 (0.015)	0.13	0.000 (0.007)	0.55
	Yes	11	0.091 (0.252)		0.044 (0.164)		0.010 (0.045)		0.001 (0.004)	
Hospitalization within 1 year	No	61	0.051 (0.139)	0.10	0.004 (0.054)	0.054	0.001 (0.014)	0.08	0.000 (0.006)	1.00
	Yes	35	0.088 (0.198)		0.024 (0.135)		0.006 (0.033)		0.000 (0.006)	
Diabetes mellitus	No	46	0.070 (0.148)	0.54	0.011 (0.064)	0.36	0.003 (0.016)	0.27	0.001 (0.009)	0.31
	Yes	50	0.051 (0.171)		0.008 (0.058)		0.002 (0.014)		0.000 (0.005)	
Diabetes with end-organ damage	No	54	0.064 (0.155)	0.48	0.015 (0.072)	0.12	0.004 (0.020)	0.10	0.000 (0.007)	0.82
	Yes	42	0.051 (0.173)		0.003 (0.053)		0.001 (0.013)		0.000 (0.008)	
Hospitalization within 1-year follow up	No	64	0.052 (0.167)	0.22	0.007 (0.062)	0.16	0.002 (0.014)	0.12	0.000 (0.005)	0.19
	Yes	31	0.074 (0.158)		0.024 (0.070)		0.006 (0.017)		0.000 (0.017)	
ER visit within 1-year follow up	No	67	0.057 (0.124)	0.18	0.010 (0.060)	0.62	0.003 (0.014)	0.68	0.000 (0.003)	0.002[a]
	Yes	28	0.105 (0.192)		0.009 (0.127)		0.002 (0.034)		0.005 (0.027)	

[a] $p < 0.05$.

Note. ABX, antibiotics; BMI, body mass index; CC, correlation coefficient; CCI, Charlson comorbidity index; ER, emergency room; HD, hemodialysis; IQR, interquartile range; OTU, operational taxonomic unit; PPI, proton pump inhibitor.

Table 4 Correlations between laboratory parameters and abundance of major Proteobacteria OTUs.

		Proteobacteria (phylum)				1111294 (*Escherichia-Shigella*)			114510 (Escherichia)			813217 (*Klebsiella*)		
		n	Median	IQR	p value	Median	IQR	p value	Median	IQR	p value	Median	IQR	p value
Albumin (median: 4 g/dL)	< Median	43	0.095	0.232	0.036[a]	0.015	0.135	0.10	0.004	0.033	0.06	0.001	0.016	0.78
	≥ Median	53	0.053	0.089		0.004	0.058		0.001	0.014		0.000	0.005	
Albumin (normal range: ≥ 3.5 g/dL)	<3.5	7	0.168	0.235	0.045[a]	0.062	0.230	0.06	0.016	0.055	0.06	0.000	0.048	0.71
	≥3.5	89	0.057	0.156		0.008	0.059		0.002	0.014		0.000	0.005	
Hemoglobin (median: 11.2 g/dL)	< Median	47	0.051	0.124	0.15	0.003	0.042	0.007[a]	0.001	0.014	0.014[a]	0.001	0.015	0.14
	≥ Median	49	0.073	0.189		0.024	0.135		0.006	0.033		0.000	0.005	
URR (median: 0.72)	< Median	37	0.045	0.139	0.24	0.004	0.045	0.09	0.001	0.012	0.13	0.000	0.004	0.91
	≥ Median	59	0.073	0.160		0.016	0.078		0.004	0.018		0.000	0.014	
Kt/V (median: 1.53)	< Median	48	0.036	0.203	0.50	0.005	0.058	0.31	0.001	0.029	0.38	0.000	0.003	0.28
	≥ Median	48	0.073	0.152		0.020	0.062		0.005	0.015		0.001	0.023	
nPCR (median: 1.2 g/kg/day)	<1.2	45	0.088	0.238	0.015[a]	0.016	0.135	0.010[a]	0.004	0.037	0.008[a]	0.000	0.004	0.68
	≥1.2	51	0.045	0.120		0.003	0.043		0.001	0.010		0.000	0.008	
BUN (median: 78 mg/dL)	< Median	47	0.088	0.228	0.003[a]	0.024	0.135	0.015[a]	0.006	0.036	0.013[a]	0.000	0.017	0.38
	≥ Median	49	0.024	0.102		0.006	0.043		0.001	0.010		0.000	0.003	
Ferritin (median: 332 ng/mL)	< Median	48	0.075	0.252	0.10	0.020	0.160	0.032[a]	0.005	0.045	0.049[a]	0.000	0.006	0.28
	≥ Median	48	0.039	0.118		0.004	0.043		0.001	0.013		0.001	0.007	
iPTH (normal range: 300 pg/mL)	<300	60	0.082	0.151	0.06	0.024	0.061	0.048[a]	0.006	0.015	0.043[a]	0.001	0.005	0.60
	≥300	36	0.025	0.181		0.003	0.042		0.001	0.028		0.000	0.013	
hsCRP (normal range: < 0.3 mg/dL)	<0.3	43	0.053	0.115	0.19	0.006	0.060	0.48	0.001	0.014	0.36	0.000	0.004	0.73
	≥0.3	53	0.074	0.189		0.012	0.102		0.003	0.031		0.000	0.015	

[a] $p < 0.05$.

Note. BUN, blood urea nitrogen; hsCRP, high-sensitivity C-reactive protein; iPTH, intact parathyroid hormone; IQR, interquartile range; nPCR, normalized protein catabolic rate; OTU, operational taxonomic unit; URR, urea reduction ratio.

of *Bifidobacterium*, and a randomized controlled trial showed that decreased consumption of advanced glycation end products decreases *Bididobacterium animalis*.[51] Dairy food has high phosphorous content and is not recommended for HD patients. Interestingly, a correlation between *Bifidobacterium* and *Blautia* was found in healthy controls, but not among HD cases, which may reflect that HD cases consume fewer dairy products. Another recent study about body weight among HD cases showed that alpha diversity positively correlated with BMI, which is consistent with results of the present study; and *Bifidobacterium* and butyrate-producing bacteria decreased in obese patients.[52] Guidelines for nutrition in HD patients make no special mention of a plant-based protein diet, although they encourage fruits and vegetables, which may help to decrease body weight.[7] The abundance of other important pathogens among HD cases included *Staphylococcus* and *C. difficile*.[53,54] The overall abundance of pathogens was low but was still higher among HD cases than that in controls.

A very recent study also conducted in Taiwan analyzing HD patients showing different results compared to our study.[55] In their study, the most abundant genus in HD patients was *Bacteroides*, but *Blautia* is the most abundant genus in our result. Interesting, high abundance in *Blautia producta* was found in peritoneal dialysis group of their cohort. Moreover, in their LEfSe analysis, phyla *Proteobacteria* (LDA score >4) and *Verrucomicrobia* were predominant in HD patients, but no detailed analysis was performed for *Proteobacteria* in their study.

This study has several limitations. First, the accuracy of species identification is limited with V3/V4 sequencing, thus, comparison at the genus level was performed for most OTUs. Whether full-length 16s rRNA sequencing could provide more information warrants further investigation.[40] We analyzed several samples with high abundance of genus *Klebsiella* and found that over 90% of genus *Klebsiellla* were *K. pneumoniae*. However, full-length analysis still could not differentiate *E. coli* from *Shigella* based on 16s rRNA sequencing. Secondly, we used the Greengenes database and OTU for analysis, while newer databases are available with ASV-based analysis. However, in the study of Martinson et al. about *Enterobacteriaceae*,[37] OTU-based analysis was slightly more accurate than ASV-based analysis. Third, we only collected stool samples once, but we had the strength of deep phenotyping and long-term follow up. Fourth, we did not analyze the food habits of each individual, which may reflect possible correlates to the results of microbiota analysis. Fifth, HD patients had special dietary guidelines which may strongly affect their microbiota. Moreover, age and sex difference between control and HD cases might affect the result of microbiota composition compared to healthy group, but the analysis within HD group is not affected by the difference. Age and sex are not associated with abundance of *Proteobacteria* among HD group.

Conclusion

The abundance of pathogens is low among HD patients and healthy controls, but abundance still remains higher among HD patients. Alpha diversity in the HD population is not lower than that of healthy controls but composition of the microbiota is notably different in the two groups according to beta diversity, due mainly to decreased *Bacteriodetes* and increased *Firmicutes* and *Proteobacteria*. HD patients have a higher abundance of *Proteobacteria* in spite of reserved diversity, but no correlations with clinical factors is noted. Genus ID 813217 (*Klebsiella*) is associated with emergency room visits during 1-year follow up after hospitalization. Deeper microbiota analyses for *Enterobacteriaceae* are necessary. Whether change in dietary components can help to decrease mortality among dialysis population warrants further research.

Declaration of competing interest

The authors declare that they have no competing interests.

Funding

This study was funded primarily by the Far Eastern Memorial Hospital-National Yang Ming Chiao Tung University Joint Research Program to Dr. Hon-Yen Wu and Dr. Yi-Tsung Lin (108DN11). Other supports included the research grant to Dr. Hon-Yen Wu from the National Health Research Institutes, Taiwan (NHRI-EX111-11026PI), and research grants to Dr. Chun-Hsing Liao from the Ministry of Science and Technology, Taiwan (MOST-108-2314-B-418-008) and the Far Eastern Memorial Hospital, New Taipei City, Taiwan (FEMH-2018-PI20180002).

Author contributions

H-YW, Y-TL, and C-HL conceived and designed the study. All authors were responsible for the acquisition, analysis, and interpretation of the data. All authors accessed and verified the data. H-YW, W-CT, H-SH, and C-HL contributed to the statistical analysis. H-YW, Y-LC, S-PH, Y-SP, and C-HL provided administrative, technical, and material support. H-YW, Y-TL, M-JK, and C-HL contributed to the acquisition of funding for the study. H-YW, J-YY, M—FP, S-PH, Y-SP, and C-HL supervised the study. H-YW, Y-TL, and C-HL drafted the manuscript. All authors critically revised the manuscript and approved the final version of the manuscript. H-YW and Y-TL contributed equally as first authors to this work.

Acknowledgments

Ethics statement

The study protocol was approved by the Far Eastern Memorial Hospital Institutional Review Board (107027-F, 107140-E). All included patients and healthy controls provided signed informed consent to participate in the study.

Data availability

Deidentified data will be made available for researchers who provide a methodologically sound proposal upon reasonable request to the corresponding author (https://

zenodo.org/record/6777118#.Yr4m1nZBwfk; https://doi.org/10.5281/zenodo.6777117).

References

1. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *N Engl J Med* 2016;**375**:2369—79.
2. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021;**19**:55—71.
3. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe* 2012;**12**:611—22.
4. Asnicar F, Berry SE, Valdes AM, Nguyen LH, Piccinno G, Drew DA, et al. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. *Nat Med* 2021;**27**:321—32.
5. Green JE, Davis JA, Berk M, Hair C, Loughman A, Castle D, et al. Efficacy and safety of fecal microbiota transplantation for the treatment of diseases other than Clostridium difficile infection: a systematic review and meta-analysis. *Gut Microb* 2020;**12**:1—25.
6. Chung CS, Chang PF, Liao CH, Lee TH, Chen Y, Lee YC, et al. Differences of microbiota in small bowel and faeces between irritable bowel syndrome patients and healthy subjects. *Scand J Gastroenterol* 2016;**51**:410—9.
7. Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis* 2020;**76**: S1—107.
8. Dalrymple LS, Johansen KL, Chertow GM, Cheng SC, Grimes B, Gold EB, et al. Infection-related hospitalizations in older patients with ESRD. *Am J Kidney Dis* 2010;**56**:522—30.
9. Ko MJ, Tsai WC, Peng YS, Hsu SP, Pai MF, Yang JY, et al. Altered monocytic phenotypes are associated with uraemic pruritus in patients receiving haemodialysis. *Acta Derm Venereol* 2021; **101**:adv00479.
10. Wu HY, Huang JW, Tsai WC, Peng YS, Chen HY, Yang JY, et al. Prognostic importance and determinants of uremic pruritus in patients receiving peritoneal dialysis: a prospective cohort study. *PLoS One* 2018;**13**:e0203474.
11. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, et al. Chronic kidney disease alters intestinal microbial flora. *Kidney Int* 2013;**83**:308—15.
12. Cigarran Guldris S, Gonzalez Parra E, Cases Amenos A. Gut microbiota in chronic kidney disease. *Nefrologia* 2017;**37**: 9—19.
13. Crespo-Salgado J, Vehaskari VM, Stewart T, Ferris M, Zhang Q, Wang G, et al. Intestinal microbiota in pediatric patients with end stage renal disease: a Midwest Pediatric Nephrology Consortium study. *Microbiome* 2016;**4**:50.
14. Simoes-Silva L, Araujo R, Pestana M, Soares-Silva I, Sampaio-Maia B. The microbiome in chronic kidney disease patients undergoing hemodialysis and peritoneal dialysis. *Pharmacol Res* 2018;**130**:143—51.
15. Suzuki M, Satoh N, Nakamura M, Horita S, Seki G, Moriya K. Bacteremia in hemodialysis patients. *World J Nephrol* 2016;**5**: 489—96.
16. Ko MJ, Peng YS, Wu HY. Uremic pruritus: pathophysiology, clinical presentation, and treatments. *Kidney Res Clin Pract* 2022. Online ahead of print.
17. Tancrede CH, Andremont AO. Bacterial translocation and gram-negative bacteremia in patients with hematological malignancies. *J Infect Dis* 1985;**152**:99—103.
18. Donskey CJ. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clin Infect Dis* 2004;**39**:219—26.
19. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2012;**55**:905—14.
20. Bossola M, Sanguinetti M, Scribano D, Zuppi C, Giungi S, Luciani G, et al. Circulating bacterial-derived DNA fragments and markers of inflammation in chronic hemodialysis patients. *Clin J Am Soc Nephrol* 2009;**4**:379—85.
21. Wang F, Jiang H, Shi K, Ren Y, Zhang P, Cheng S. Gut bacterial translocation is associated with microinflammation in end-stage renal disease patients. *Nephrology* 2012;**17**:733—8.
22. Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, et al. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 2002;**68**:5445—51.
23. Matsuki T, Watanabe K, Fujimoto J, Takada T, Tanaka R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol* 2004;**70**:7220—8.
24. Ko MJ, Huang JW, Wu HY, Tsai WC, Hsu LY, Tsai PH, et al. Risk of skin cancer among patients with chronic kidney disease treated with ultraviolet B phototherapy for uraemic pruritus: a nationwide cohort study. *Acta Derm Venereol* 2021;**101**:adv00390.
25. Wang J, Wu HY, Chien KL. Cardioprotective effects of dipeptidyl peptidase-4 inhibitors versus sulfonylureas in addition to metformin: a nationwide cohort study of patients with type 2 diabetes. *Diabetes Metab* 2021;**48**:101299.
26. Depner TA, Daugirdas JT. Equations for normalized protein catabolic rate based on two-point modeling of hemodialysis urea kinetics. *J Am Soc Nephrol* 1996;**7**:780—5.
27. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chron Dis* 1987;**40**:373—83.
28. Andrews S. *Fast QC: a quality control tool for high throughput sequence data*. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc. [Accessed 14 April 2022].
29. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 2014;**30**:614—20.
30. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460—1.
31. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**: 335—6.
32. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 2006;**72**:5069—72.
33. Wu IW, Lin CY, Chang LC, Lee CC, Chiu CY, Hsu HJ, et al. Gut microbiota as diagnostic tools for mirroring disease progression and circulating nephrotoxin levels in chronic kidney disease: discovery and validation study. *Int J Biol Sci* 2020;**16**:420—34.
34. Sampaio-Maia B, Simoes-Silva L, Pestana M, Araujo R, Soares-Silva IJ. The role of the gut microbiome on chronic kidney disease. *Adv Appl Microbiol* 2016;**96**:65—94.
35. Hoarau G, Mukherjee PK, Gower-Rousseau C, Hager C, Chandra J, Retuerto MA, et al. Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial crohn's disease. *mBio* 2016;**7**:e01250. 16.
36. Weng MT, Chiu YT, Wei PY, Chiang CW, Fang HL, Wei SC. Microbiota and gastrointestinal cancer. *J Formos Med Assoc* 2019;**118**(Suppl 1):S32—41.
37. Martinson JNV, Pinkham NV, Peters GW, Cho H, Heng J, Rauch M, et al. Rethinking gut microbiome residency and the Enterobacteriaceae in healthy human adults. *ISME J* 2019;**13**: 2306—18.

38. Yu D, Banting G, Neumann NF. A review of the taxonomy, genetics, and biology of the genus Escherichia and the type species Escherichia coli. *Can J Microbiol* 2021;**67**:553—71.
39. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep* 2013;**5**:58—65.
40. Johnson JS, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, et al. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun* 2019;**10**:5029.
41. Adeolu M, Alnajar S, Naushad S. R SG. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. *Int J Syst Evol Microbiol* 2016;**66**:5575—99.
42. Tamburini FB, Andermann TM, Tkachenko E, Senchyna F, Banaei N, Bhatt AS. Precision identification of diverse bloodstream pathogens in the gut microbiome. *Nat Med* 2018;**24**:1809—14.
43. Martin RM, Cao J, Brisse S, Passet V, Wu W, Zhao L, et al. Molecular epidemiology of colonizing and infecting isolates of Klebsiella pneumoniae. *mSphere* 2016;**1**:e00261. 16.
44. Huang YT, Jiang JY, Hsu MS, Hsu HS, Liao CH, Hsueh PR. The prevalence of rectal carriage of Klebsiella pneumoniae amongst diabetic patients and their clinical relevance in Taiwan: a five-year prospective study. *J Microbiol Immunol Infect* 2018;**51**:510—8.
45. Wang IK, Lai HC, Yu CJ, Liang CC, Chang CT, Kuo HL, et al. Real-time PCR analysis of the intestinal microbiotas in peritoneal dialysis patients. *Appl Environ Microbiol* 2012;**78**:1107—12.
46. Gomez-Gallego C, Garcia-Mantrana I, Martinez-Costa C, Salminen S, Isolauri E, Collado MC. The microbiota and malnutrition: impact of nutritional status during early life. *Annu Rev Nutr* 2019;**39**:267—90.
47. Benitez-Paez A, Gomez Del Pugar EM, Lopez-Almela I, Moya-Perez A, Codoner-Franch P, Sanz Y. Depletion of Blautia species in the microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. *mSystems* 2020;**5**:e00857. 19.
48. Ozato N, Saito S, Yamaguchi T, Katashima M, Tokuda I, Sawada K, et al. Blautia genus associated with visceral fat accumulation in adults 20-76 years of age. *NPJ Biofilms Microbiomes* 2019;**5**:28.
49. Dupuis L, Brown-Tortorici A, Kalantar-Zadeh K, Joshi S. A Mini review of plant-based diets in hemodialysis. *Blood Purif* 2021;**50**:672—7.
50. Wu BS, Wei CH, Yang CY, Lin MH, Hsu CC, Hsu YJ, et al. Mortality rate of end-stage kidney disease patients in Taiwan. *J Formos Med Assoc* 2022;**121**(Suppl 1):S12—9.
51. Yacoub R, Nugent M, Cai W, Nadkarni GN, Chaves LD, Abyad S, et al. Advanced glycation end products dietary restriction effects on bacterial gut microbiota in peritoneal dialysis patients; a randomized open label controlled trial. *PLoS One* 2017;**12**:e0184789.
52. Lin TY, Wu PH, Lin YT, Hung SC. Characterization of gut microbiota composition in hemodialysis patients with normal weight obesity. *J Clin Endocrinol Metab* 2020;**105**:2006—14.
53. Ramesh MS, Yee J. Clostridioides difficile infection in chronic kidney disease/end-stage renal disease. *Adv Chron Kidney Dis* 2019;**26**:30—4.
54. Krezalek MA, Hyoju S, Zaborin A, Okafor E, Chandrasekar L, Bindokas V, et al. Can methicillin-resistant Staphylococcus aureus silently travel from the gut to the wound and cause postoperative infection? Modeling the "trojan horse hypothesis. *Ann Surg* 2018;**267**:749—58.
55. Shivani S, Kao CY, Chattopadhyay A, Chen JW, Lai LC, Lin WH, et al. Uremic toxin-producing Bacteroides species prevail in the gut microbiota of Taiwanese CKD patients: an analysis using the new taiwan microbiome baseline. *Front Cell Infect Microbiol* 2022;**12**:726256.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2022.12.001.