

Original Article

Potential clinical usefulness of gut microbiome testing in a variety of clinical conditions

Karl J. Morten^{a,1}, Eleonora Staines-Urias^{a,1}, Julian Kenyon^{b,*}^a Nuffield Department of Women's and Reproductive Health, University of Oxford, United Kingdom^b The Dove Clinic for Integrated Medicine, The Old Brewery, High Street, Twyford, Winchester, United Kingdom

1. Introduction

The gut microbiome comprises the community of microorganisms in the intestinal tract. Over the last five years, interest in the gut microbiome has grown considerably driven by new techniques in DNA sequencing allowing for characterisation of gut bacteria and the recognition of the potential impact the microbiome may have on health [1,2]. The large intestine has the highest number of microbial organisms, with less found in the more hostile low-pH environment of the small intestine. The large intestine is dominated by anaerobic bacteria which survive and thrive by anaerobically digesting our food [3–5]. The gut microbiome has coevolved with humans to match our modern lifestyles [6] and is beneficial for our health, supplying essential nutrients, synthesizing vitamins (i.e. vitamin K) and facilitating digestion of undigested carbohydrates [7–9]. Furthermore, bacteria also help maintain the integrity of the mucosal barrier by preventing antigens and pathogens entering the gut mucosa [10,11].

In healthy adults, 80% of the identified faecal microbiota can be classified into three dominant phyla: Bacteroides, Firmicutes and Actinobacteria. In general terms, the Firmicutes to Bacteroides ratio is regarded to be of significant relevance in the human gut microbiota composition. High Firmicutes and low Bacteroides usually correlates with a healthy diverse microbiome and reflects a largely plant-based diet. In unhealthy microbiomes the opposite is the case [12,13]. Alterations in the composition of the microbiome has the potential to significantly impact on our health and wellbeing. One of the side effects of antibiotic use is a change in gut microflora that allows overgrowth of harmful micro-organisms [14]. *Clostridium difficile*-associated diarrhoea for example is a well-recognised infection linked to previous antibiotic use [15]. Furthermore, studies on young children with a developing microbiome have shown that antibiotics are especially likely to cause long lasting changes [16–18]. Regulation of the gut flora has also been correlated with a host of inflammatory and immune conditions [19,20]. Recent changes in lifestyle including reduced exposure to pathogens in early life, dietary changes to a high intake of carbohydrates and fats from processed foods and reduced dietary fibre have been proposed to

play a role in the rise of inflammatory conditions such as inflammatory bowel disease (IBS/D) and Crohn's disease [19,20]. The microbiome has been shown to have profound effects in the development of gut-associated lymphoid tissue, differentiation of gut immune cells and production of immune mediators such as IgA's and microbial defence peptides [21]. Recent research suggests that an altered microbiome may play a role in a wide range of disorders including Parkinson's disease [22,23] chronic liver disease [24,25], myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [26,27] and also impact cancer patient recovery after treatments such as chemotherapy and radiotherapy (Reviewed in [28]).

In this study we investigated microbiome diversity in a range of conditions including cancer, ME/CFS, inflammatory bowel disease (IBS), and obesity among others. Samples from 39 patients in five clinical groups representing a variety of diagnoses and a group of asymptomatic individuals were analysed to obtain relative proportions of bacterial taxa and to quantify the overall diversity of the gut microbiome relative to the average British population represented by data from the Twins UK and British Gut studies. Comparisons of microbiome data from the diseased and healthy individuals in our sample were carried out for bacterial relative abundance and a diversity score.

2. Materials and methods

2.1. Sample collection and gut bacterial genotyping

Stool samples were collected by the participants and sent for analysis to the Map My Gut service at St Thomas Hospital (Fig. S1). A total of 39 patients followed the protocol, and their samples were included in this study.

As previously described, bacterial 16S ribosomal RNA (rRNA) gene sequencing of DNA isolated from faecal samples was carried out using an established protocol. DNA was extracted from the stool samples and deep sequencing of the V4 region of the 16SRNA carrier out with demultiplexing of sequencing reads. Sequences were grouped into operational taxonomic units (OTUs, $\geq 97\%$ similarity) using the

* Corresponding author at: The Dove Clinic, The Old Brewery, Winchester, Hampshire SO21 1RG, United Kingdom.

E-mail address: jkenyon@doveclinic.com (J. Kenyon).¹ These authors contributed equally to this work.

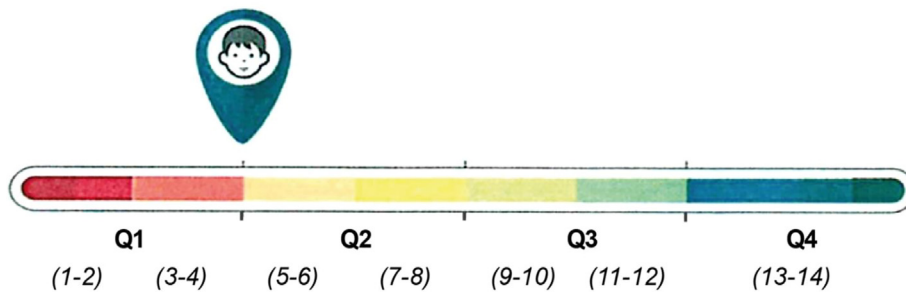


Fig. 1. Microbiome diversity scoring. The grading score was derived from a relative comparison of the diversity in the Map My Gut database representing the general British population. The alpha-diversity distribution was first divided in quartiles, with the first three quartiles being further divided into two equal segments. Each of the resulting seven segments accounts for two units of the score, giving the diversity score a range between 0 and 14, with a higher score meaning higher microbiome diversity.

Table 1

Basic demographic characteristics, average relative bacterial abundance at phylum, family, and genus levels, and average diversity score for the six clinical groups.

Demographics	Cancer N = 15	ME/CFS N = 7	IBS N = 4	Obesity N = 3	Others N = 5	Controls N = 5	p-value*
Age, years (median, IQR)	66 (60, 68)	58 (44, 62)	40 (21, 56)	64 (63, 71)	55 (18, 72)	54 (36, 57)	0.077
Females, N (%)	10 (66.7)	5 (71.4)	4 (100.0)	2 (66.7)	3 (60.0)	5 (100.00)	0.302
Diversity score (median)	4.0	3.0	4.5	4.0	6.0	13.0	0.006
Relative abundance – Phylum (%)							
Bacteroidetes	47.0	52.0	55.5	55.0	39.0	30.0	0.067
Firmicutes	50.0	45.0	39.5	43.0	54.0	62.0	0.067
Firmicutes/Bacteroidetes	1.1	0.9	0.7	0.8	1.4	2.1	0.065
Proteobacteria	1.1	1.5	3.9	1.8	1.7	2.2	0.705
Tenericutes	0.1	0.3	0.0	0.3	0.1	1.7	0.011
Actinobacteria	0.9	0.7	0.4	1.0	0.3	0.9	0.628
Verrucomicrobia	0.0	0.1	0.6	0.0	0.1	0.0	0.390
Relative abundance – Family (%)							
Bacteroidaceae	15.0	38.0	43.5	48.0	31.0	25.0	0.644
Barnesiellaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.237
Paraprevotellaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.026
S24-7	0.0	0.0	0.0	0.0	0.0	0.0	0.009
Rikinellaceae	0.0	1.3	6.9	3.6	2.0	4.9	0.254
Porphyromonadaceae	2.9	0.0	5.9	2.8	1.4	0.0	0.090
Paeniacilaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Enterococaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Streptococaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.583
Lachnospiraceae	13.0	16.0	15.5	22.0	12.0	23.0	0.085
Ruminococcaceae	23.0	18.0	18.0	15.0	21.0	26.0	0.096
Clostridiaceae	3.0	0.0	0.0	0.0	0.0	0.0	0.239
Peptostreptococaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Christensenellaceae	0.1	0.0	0.0	0.0	0.0	0.1	0.945
Erysipelotrichaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.017
Veillonellaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.104
Alcaligenaceae	0.0	0.0	0.8	0.0	0.0	0.0	0.925
Comamonadaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Desulfovibrionaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Anaeroplasmataceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Coriobacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.701
Relative abundance – Genus (%)							
Prevotella	3.4	6.5	0.0	0.0	0.0	0.0	0.343
Bacteroides fragilis	0.0	0.1	0.0	1.7	0.0	0.1	0.588
Bacillus	0.0	0.0	0.0	0.0	0.0	0.0	na
Lactobacillus	0.0	0.0	0.0	0.0	0.0	0.0	0.495
F.prausnitzii	5.5	5.8	5.0	4.9	9.1	10.0	0.011
Coprococcus	3.0	2.0	2.0	3.9	2.3	2.6	0.850
Lachnobacterium	0.0	0.0	0.0	0.2	0.0	0.1	0.493
Roseburia	1.1	0.8	0.3	2.3	0.5	2.3	0.077
Oxalobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.472
Bifidobacterium	0.3	0.2	0.2	0.3	0.1	0.5	0.750
Akkermansia	0.0	0.1	0.6	0.0	0.1	0.0	0.390
Methanobrevibacter	0.0	0.0	0.0	0.0	0.0	0.0	na

* p-value from ranksum test (chi squared test for sex, with exact Fisher's test p-value) comparing all disease groups combined with the controls. Na: not applicable (not present in the sample).

Greengenes 16S rRNA reference database at different levels of classification. Results were reported for the phyla, families, and genera most commonly found in the human gut microbiota. OTU-based alpha bacterial diversity was estimated for each sample.

2.2. Microbiome diversity scoring

The microbiome diversity of each sample was scored relative to the diversity distribution observed in the general British population characterised by the 'normal' microbiome composition of the Twins UK and British Gut databases (<https://mapmygut.com/>). The Twins database

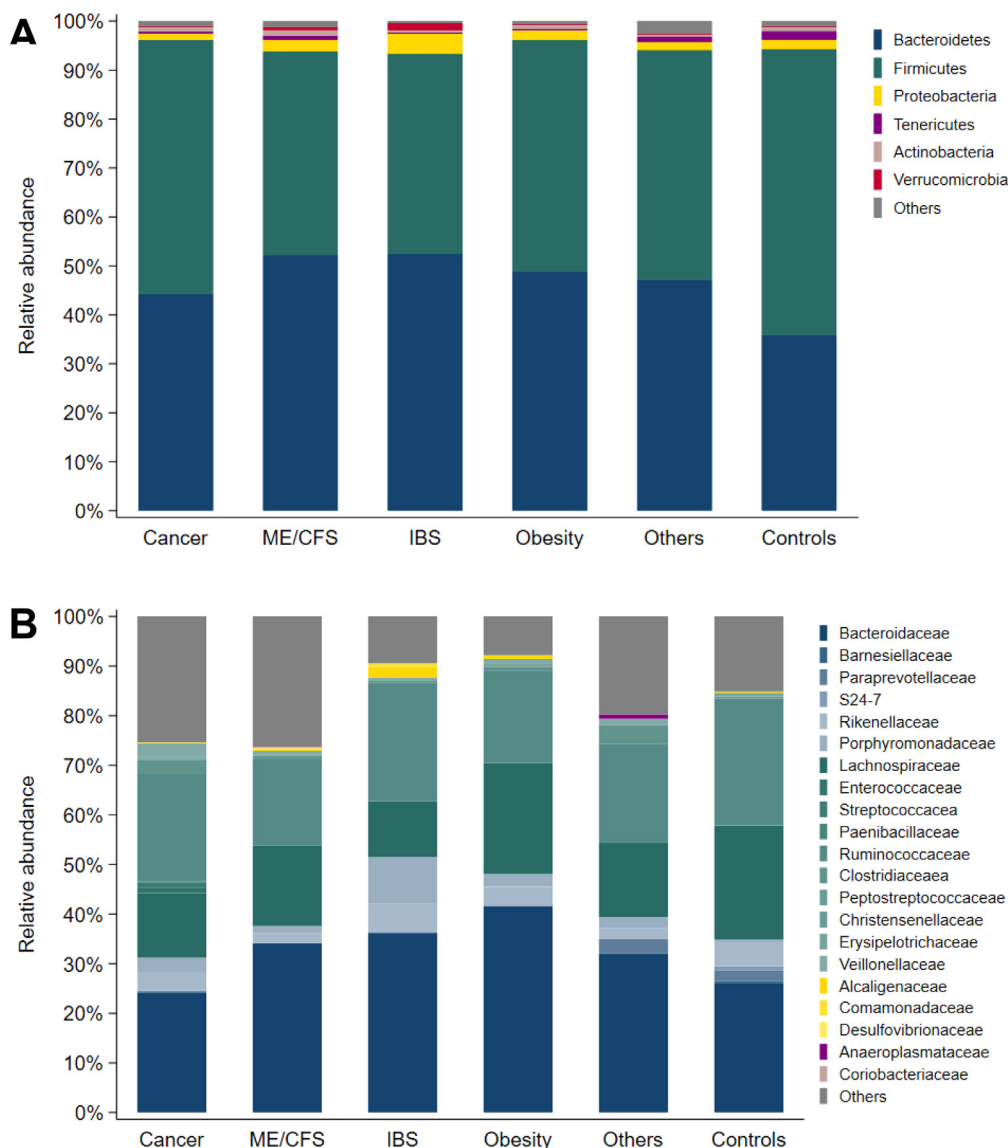


Fig. 2. A) Top panel. Percentage distribution of bacterial phyla in human gut microbiomes for five disease groups and a control group. B) Bottom panel. Percentage distribution of selected bacterial families in human gut microbiomes for five disease groups and one control group.

comprises of a total of 12,000 identical and non-identical twins from across the UK and Ireland. Female twins predominate, and the mean age is in the mid-50s. More than 3000 twins in the data base have had their gut microbiome analysed by sequencing the 16S gene found in their stools. The British Gut study, as of August 2016, comprises over 900 participants from across Britain. The population is equally distributed between women and men (56% females, 44% males), the average age is 47 years, and the average BMI is 35.6 kg/m². All participants of this study have had gut microbiome analysis.

In our study, the scoring system involved dividing the total diversity in the reference population into quartiles, the first three quartiles being further divided into two equal segments. Each of the resulting seven segments accounted for two units of the score, giving the diversity score a range between 0 and 14, a higher score meaning higher diversity (Fig. 1). Microbiome diversity has been shown to be the best assessment of the microbiome constitution, showing consistency between longitudinal samples.

2.3. Statistical analysis

Bacterial relative abundance at the phylum, family, and genus levels was compared between cases and controls using non-parametric methods (two-tailed Wilcoxon rank-sum test). Due to the small sample size, exploration of differences was first conducted comparing all combined cases ($n = 34$) against the control group ($n = 5$). Suggestive associations ($p < 0.07$) were followed with comparisons between each disease group and the controls. No adjustment for multiple testing was done. A final comparison between cases and controls was performed for the diversity score.

Statistical analyses were done using STATA 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

3. Results

3.1. Demographics of patient and healthy control groups

The cancer group comprised 15 adults aged 43–78 years old, 5 male

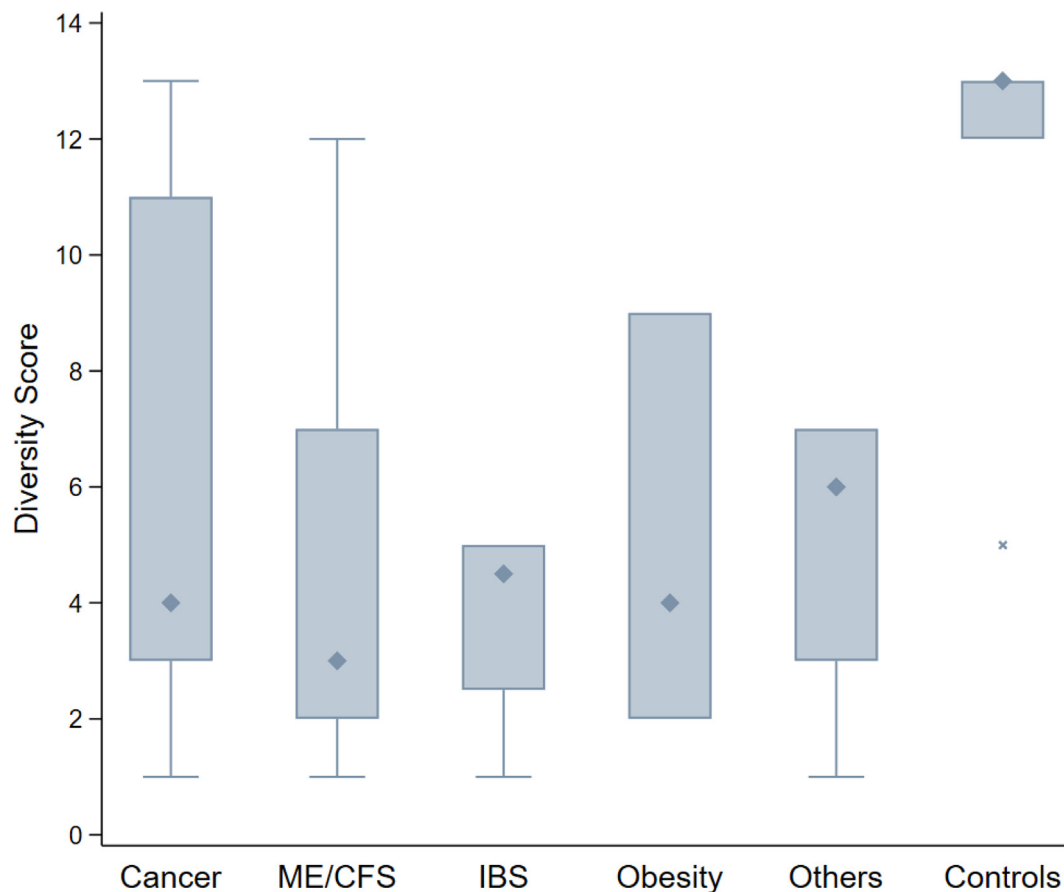


Fig. 3. Distribution of gut microbiome diversity score according to clinical group.

and 10 female, with a range of cancers including bladder, pancreas, colorectal, parapharyngeal, breast, kidney, prostate, lymphoma, glioblastoma multiforme, and malignant melanoma. This cancer collection is in keeping with patients seen in private clinics with chronic illnesses, but differs from most previously published microbiome studies which have focused primarily on colorectal cancer patients. The ME/CFS group consisted of 7 adults aged 41–68 years old, 2 male and 5 female, with a long history of the disease (> 10 years). All patients in this group conformed to the Fukuda or Canadian criteria for ME/CFS. The group of patients with IBS included 4 women aged 10–64 years. A small obesity group of 3 adults was included together with a group of 5 individuals with a range of conditions including bowel infection, thrush/cystitis, abdominal pain following surgery, and rhinitis/sinus problems.

The control group comprised 5 adult women aged 32–59 years who had been completely asymptomatic up to when they provided the sample and considered themselves healthy (Table S1).

3.2. Bacterial taxa comparisons

Table 1 and Fig. 2 show the average percentage distribution of bacterial phyla and families comprising the gut microbiome in each clinical group (individual profiles are presented in Fig. S2).

When comparing bacterial phyla, there was a difference in Tenericutes levels between cases and controls ($p = 0.011$); nevertheless, the association was lost after zeroes were removed ($p = 0.101$). There was a suggestion that Firmicutes levels were decreased ($p = 0.067$) and Bacteroidetes levels were increased ($p = 0.067$) in cases compared to controls. The difference in Firmicutes was observed for the IBS ($p = 0.049$) and ME/CFS ($p = 0.062$) disease groups, while the increase in Bacteroidetes was observed mainly for the ME/CFS patients ($p = 0.061$) compared to the control group.

At the family level, S24-7 was present only in one control ($p = 0.009$); and Erysipelotrichaceae ($p = 0.017$) and Paraprevotellaceae ($p = 0.026$) were present in two cases and two controls. Associations were lost after zeroes were removed.

Although the Ruminococcaceae family was not different between cases and controls ($p = 0.096$), *F. prausnitzii* was lower in all combined cases ($P = 0.011$), the cancer group ($p = 0.004$), and the obesity group (0.027) compared to the controls. The Lachnospiraceae family was decreased in cases ($p = 0.053$), particularly the cancer group ($p = 0.044$). There was an indication that Roseburia was decreased among cases ($p = 0.077$), driven by not-nominally significant differences for the cancer ($p = 0.066$) and the IBS ($p = 0.065$) groups.

3.3. Diversity score

Because comparison of patients' data at the genus level has proved to lack reproducibility across studies, we were interested in the performance of the diversity score approach.

Using a measure of diversity to assess the microbiome, a normal healthy gut should have a score between 10 and 11 units (<https://mapmygut.com/>). Our control group included five individuals who were symptom free and considered themselves healthy when they provided a stool sample. This group had a median diversity score of 13 units, which compares very well to the healthy reference range. In contrast, the median value of the score was 4 units for all cases combined ($p = 0.024$; Fig. 3). Cancer and ME/CFS patients had significant reduced diversity compared to the control group with medians of 4.0 ($p = 0.024$) and 3.0 units ($p = 0.014$) respectively. The diversity score was also reduced in the IBS (median 4.5 units; $p = 0.023$), obesity (median 4 units; $p = 0.047$), and miscellaneous groups (median 6 units; $p = 0.044$). None of the patients in the Study had been on Antibiotics

over the previous 12 months.

4. Discussion

This study was undertaken to determine how assessment of the microbiome can be used to help with patient treatment in general practice. For many patients with chronic conditions like the ones represented in this study, established treatment approaches are not available, with limited options available for symptom management.

In this study we make the surprising observation that gut bacterial diversity score, relative to the distribution of diversity in a healthy, adult British population, is significantly reduced in a range of chronic diseases compared to a healthy control group. The average diversity score of 13 units for the controls agrees with the average diversity score, between 10 and 11 units, of the population reference data run by Map My Gut (unpublished data).

For clinicians in UK general practice, having information about the microbiome could have an important utility in deciding on treatment options.

We consider measurement of gut microbiota diversity a potentially relevant clinical tool for the overall management of patients with chronic conditions.

5. Conclusion

Potential clinical effectiveness of the diversity score applicable to a wide range of medical conditions, has greater sensitivity and reduces multiple testing compared to bacteria taxa comparisons. Faecal microbiome testing is clinically useful and potentially defines treatment directions in a particular patient.

Conflicts of interest

JK is the owner of the Dove Clinic. KM and ESU have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.humic.2018.08.003>.

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