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Original research

Postinfective bowel dysfunction following *Campylobacter enteritis* is characterised by reduced microbiota diversity and impaired microbiota recovery

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ABSTRACT

Objectives Persistent bowel dysfunction following gastroenteritis (postinfectious (PI)-BD) is well recognised, but the associated changes in microbiota remain unclear. Our aim was to define these changes after gastroenteritis caused by a single organism, *Campylobacter jejuni*, examining the dynamic changes in the microbiota and the impact of antibiotics.

Design A single-centre cohort study of 155 patients infected with *Campylobacter jejuni*. Features of the initial illness as well as current bowel symptoms and the intestinal microbiota composition were recorded soon after infection (visit 1, <40 days) as well as 40–60 days and >80 days later (visits 2 and 3). Microbiota were assessed using 16S rRNA sequencing.

Results PI-BD was found in 22 of the 99 patients who completed the trial. The cases reported significantly looser stools, with more somatic and gastrointestinal symptoms. Microbiota were assessed in 22 cases who had significantly lower diversity and altered microbiota composition compared with the 44 age-matched and sex-matched controls. Moreover 60 days after infection, cases showed a significantly lower abundance of 23 taxa including phylum Firmicutes, particularly in the order Clostridiales and the family *Ruminococcaceae*, increased Proteobacteria abundance and increased levels of Fusobacteria and Gammaproteobacteria. The microbiota changes were linked with diet; higher fibre consumption being associated with lower levels of Gammaproteobacteria.

Conclusion The microbiota of PI-BD patients appeared more disturbed by the initial infection compared with the microbiota of those who recovered. The prebiotic effect of high fibre diets may inhibit some of the disturbances seen in PI-BD.

Trial registration number NCT02040922.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Changes in the microbiota including reduction in some Clostridial taxa and increases in Proteobacteria have been variably reported in irritable bowel syndrome (IBS).
⇒ Approximately 13% of IBS patients report a postinfectious origin (PI-IBS).
⇒ Campylobacter enteritis alters the microbiota and 14% of cases develop PI-bowel dysfunction (PI-BD) but how these are linked is unclear.

WHAT THIS STUDY ADDS

⇒ Recovery of the microbiota in PI-BD differed significantly from those whose bowel habit had returned to normal.
⇒ PI-BD was associated with a reduction in Firmicutes and increase in Proteobacterial taxa (including taxa from class Gammaproteobacteria) which persisted for >12 weeks.
⇒ Low consumption of fibre was associated with increased levels of Gammaproteobacteria.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ By indicating specific changes in microbiota in PI-BD, it will facilitate targeted manipulation of microbiota (eg, dietary fibre, probiotics or faecal microbiota transplants) to restore normal function.

BACKGROUND

Postinfectious irritable bowel syndrome (PI-IBS) is a well-recognised symptom complex occurring in about 1 in 10 of cases of enteritis[1] and may account for up to 13% of all IBS cases.[2] The risk of developing PI-IBS appears to be greater in protozoan and bacterial enteritis as compared with viral gastroenteritis.[1] The associated activation of the immune system is an important strategy for pathogens infecting the gut since it suppresses the resident microbiota, particularly anaerobes, allowing overgrowth of the infecting pathogen[3] as well as other potentially pathogenic taxa. The reduction in anaerobic metabolites, including short chain fatty acids (SCFAs) and secondary bile acids, raises the colonic luminal pH[4 5] and reduces colonisation resistance, typically allowing an overgrowth of both the pathogen and *Proteobacteriacae*, including facultative anaerobes such as *Enterobacteriacae*.[6]

The definition of a healthy microbiota is complicated due to the large compositional variation between subjects.[7] Nonetheless, parameters such as high diversity and gene richness, abundance of SCFA production and resilience are considered to

be relevant markers of health.[7 8] A resilient microbiota is able to return to its original composition after facing a perturbation, such an infection, whereas non-resilient microbiota may shift its composition permanently to a new altered state of dysbiosis.[8 9] It has been shown that in healthy subjects, the gut microbiota recovers rapidly after a non-inflammatory diarrhoea such as that induced by osmotic laxatives like macrogols, when the colonic lumen is alkalinised.[10] This has been associated with a profound depletion of anaerobes and an increase in Proteobacteria but the observed dysbiosis was largely reversed after 14 days.[11] However, what host or dietary factors determine the recovery of the microbiota after an inflammatory diarrhoea is unclear, while the potential lack of resilience has not yet been characterised in patients developing PI-IBS. Most studies of PI-IBS combine patients infected by varying pathogens, which introduces considerable variability since each pathogen has unique features. Our work has attempted to reduce this source of variability by focusing on a single pathogen, *Campylobacter jejuni,*[12] one of the most common causes of bacterial gastroenteritis in Europe.[13]

Previous pilot studies have shown that PI-IBS following Campylobacter enteritis could be characterised by an index of microbial dysbiosis based on 27 taxa, which distinguished PI-IBS from controls. It was characterised by a 12-fold increase of Bacteroidetes taxa in patients, and a 35-fold reduction in the strict anaerobes characterised as uncultured Clostridia compared with healthy controls.[14] These findings were replicated in a meta-analysis including an additional PI-IBS group.[15] Furthermore, similar findings were seen in those who had persistent bowel dysfunction (BD) after *C. jejuni* enteritis but who did not meet Rome criteria (postinfectious BD, PI-BD).[14]

The aim of this study was to define in more detail and with greater patient numbers the serial changes in microbiota recovery over the 3 months following a culture-proven infectious gastroenteritis due to *C. jejuni*. We compared the microbiota composition, bowel symptoms, stool form and dietary habits and potential predisposing factors of PI-BD patients with controls whose bowels had returned to normal within 3 months of infection. Previous studies indicated that PI-BD is more common than PI-IBS but has similar bowel disturbance, namely persistent diarrhoea, the main difference being lack of pain.[16] We hypothesised that there would be a difference between those with PI-BD both in their response to infection and during the recovery period. More specifically, we expected to see an initial loss of microbial diversity for all patients, with a greater disturbance in those who went on to develop persistent BD. We aimed to identify these indicators of non-resilience leading towards PI-IBS-associated microbiota.

MATERIALS AND METHODS

Subjects and study design

This was a single-centre cohort study of patients who tested positive for *Campylobacter* spp. in the Public Health England Laboratory in Nottingham. The General Data Protection Regulations and heavy workload meant that potential participants were informed of their diagnosis and invited to participate by weekly mail out. Only once the subject had made contact could we then negotiate a date for a visit. This meant that the first visit was several weeks after the initial diagnosis. Figure 1 shows recruitment details. The clinical study included all 155 eligible subjects who provided clinical details of their illness, psychological parameters and bowel function.

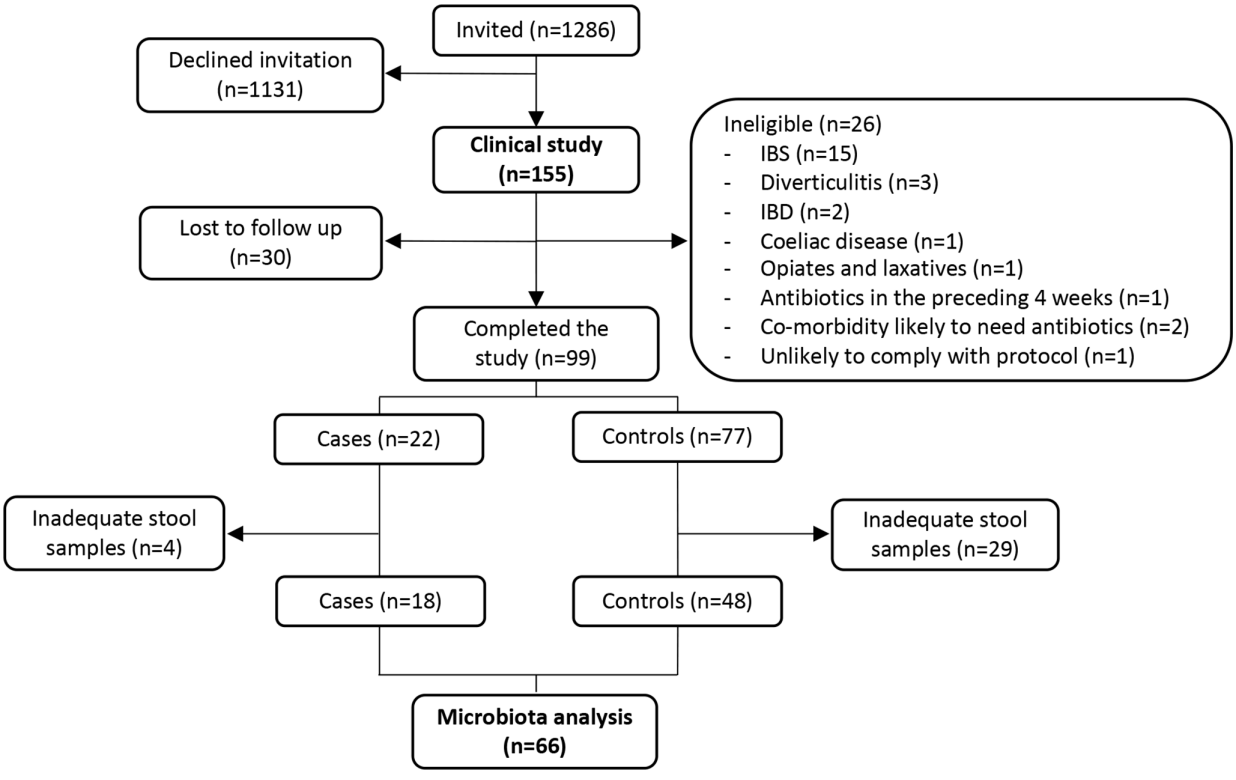

Figure 1 CONSORT diagram. The 48 mechanistic controls were chosen because they provided the most complete set of stool samples. The mechanistic study was confirmed to be unbiased from the larger clinical study by demonstrating there were no significant differences in demographics, psychological scores nor markers of initial illness severity (online supplemental tables S6–7). CONSORT, Consolidated Standards of Reporting Trials; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome.

The first stool sample was collected as early as possible following microbiological diagnosis and further samples were collected 6 and 12 weeks after diagnosis. Our previous study indicated that symptoms persisting at 12 weeks would be long-lasting (ie, >6 months).[16] However, administrative delays meant that the first faecal sample was collected at visit 1 which was a mean of 46 days (range 17–93) and the final sample at visit 3 was collected at mean 97 days (range 57–160) from the start of symptoms.

At visit 1, eligibility was confirmed and written informed consent obtained. Demographics and current bowel habits were recorded, and all completed the Hospital Anxiety and Depression Scale[17] and the Patient Health Questionnaire-12 Somatic Symptom Scale (PHQ-12 SS).[18] They were also asked about features of the acute illness with markers of severity including rectal bleeding, vomiting, weight loss, duration of time off normal activities and any antibiotic treatment.

Patients were asked to collect stool samples for each visit (see online supplemental methods for more details). If visit 1 occurred within 5 weeks of diagnosis, patients were asked to return for visit 2 at 6 weeks (typically 1 week later) to provide a further stool sample. At visit 3, 12 weeks after diagnosis, patients were asked to complete a questionnaire on their bowel symptoms from the past week and provide a further stool sample.

Dietary data

We analysed 7-day completed food diaries at visit 2 and visit 3 of 19 cases who returned an adequate food diary and age-matched and sex-matched them to 31 controls. Dietary data from each recording was manually entered into a dietary software package: Dietplan 7 (Forestfield Software V.7.00.64) for nutrient analysis. Macronutrient and micronutrient analysis was based on McCance and Widdowson's food composition data, UK. A cut-off for energy intake was set for energy levels of ≤800 kcal or ≥4500kcal/day to remove implausible reported intake.

Stool measurements

Stool SCFA concentrations and dry weights were measured in visit 2 and visit 3 samples from 14 cases and 23 controls who provided adequate additional faecal samples. Samples were analysed using gas chromatography-mass spectrometry as described previously.[19]

Microbiota analysis

Faecal DNA was extracted using a validated method.[20 21] In short, cells were lysed using a bead beater (MagNA lyser, Roche diagnostics, Indianapolis,USA). Ammonium acetate, isopropanol and centrifugation were used to precipitate the proteins and nucleic acid. A commercially available kit (QIAamp DNA Mini Kit, Qiagen, Venlo, Netherlands) was used to clean the DNA by removing the RNA and proteins. The DNA was eluted in 200 μL nuclease-free water.

Microbiota composition was analysed with the Illumina MiSeq platfrom amplifying the V3-V4 hypervariable region of the 16S rRNA gene.[22] The obtained sequence reads (on average 88 213 per sample) were prepossessed with the Mare R package[23] ProcessReads and TaxonomicTable functions the use of these is detailed in online supplemental methods. We used the SILVA 16S rRNA reference database (version 115) for taxonomic assignment. After preprocessing, there were on average 64 385 reads per sample (ranging from 28 680 to 351 004). The reads have been deposited to ENA (PRJEB52306).

Outcome measures

Clinical study

The primary outcome was the proportion of patients with BD 12 weeks after laboratory report of infection, hereafter described as PI-BD. This was defined by answering 'no' to the question 'have your bowels returned to normal since your Campylobacter infection?' at visit 3. We used this simpler measure rather than the Rome definition since we knew from previous studies[16] that a substantial number of those who complained of persistently altered bowel habit did not meet Rome criteria, mainly because they did not experience significant pain, despite having all the other key symptoms. Secondary outcomes included number of patients meeting Rome III criteria for IBS (other than 6-month duration) to allow easier comparison with other studies. We also examined the influence of age, gender, psychological factors and severity of initial illness on the risk of developing PI-BD.

Microbiota analysis

The primary outcome was microbiota recovery as assessed from diversity, richness and the abundance of key bacterial taxa. Secondary outcomes were associations between dietary components and SCFA concentrations and stool water content.

Statistical analysis and sample size calculation

Clinical study

Data are represented by mean (SD) and non-symmetrical data by median (IQR). All statistical analyses were performed by using R (V.3.6.1) and GraphPad Prism (V.8.2.1). Normality was tested with D'Agostino's K^2 test. Statistical differences of markers of disease severity were tested using Fisher's exact test or unpaired t-test, depending on normality.

We originally planned to recruit 450 participants aiming for 80% power to detect an increase in PI-BD to 39% in those taking antibiotics from 25% in those not taking antibiotics, assuming that 30% took antibiotics. However, the end of funding was reached with only 155 subjects recruited so we were substantially underpowered for this endpoint. However, the mechanistic study was larger than expected, being one of the largest in-depth study of the changes in microbiota following Campylobacter enteritis.

Microbiota analysis

To exclude biases due to antibiotics consumption, we excluded all samples collected from those subjects who consumed antibiotics (n=18, 9 each in cases and controls) until 60 days after reported infection. All taxonomic ranks from phylum down to genus level taxa were used for statistical testing. Microbial alpha-diversity was assessed using inverse Simpson diversity index using amplicon sequence variance (ASV)-level data. There was no significant correlation between alpha diversity and sample read counts (see online supplemental methods). Principal co-ordinate analysis (PCoA) with Bray-Curtis dissimilarities was used to visualise microbial beta-diversity using ASV-level data. The statistical difference between groups in the PCoA was tested using permanova and using vegan package function adonis. To test differences in the bacterial abundance between cases and controls and the associations between the bacterial taxa and nutritional components and SCFA amounts, generalised linear mixed models were used (detailed in online supplemental methods). Here, the read number for each sample was used as an offset and subject's age was used as a confounding factor. This was also supported by Spearman correlation testing. The obtained p values were adjusted for multiple testing with the

Table 1 Patient demographics at baseline

	Cases	Controls	P value
Subjects	22	77	–
Age, median (IQR)	57 (41–64)	62 (48–71)	0.05
Female, n (%)	18 (82)	33 (65)	0.002
PHQ-12 SS, median (IQR)	5 (3–6)	2 (1–4)	0.002
HADS-A, median (IQR)	5 (4–10)	5 (3–7)	0.22
HADS-D, median (IQR)	4 (1–6)	3 (1–5)	0.67
Weekly stool frequency preinfection, median (IQR)	7 (7–7)	7 (7–14)	0.31
Weekly stool frequency postinfection, median (IQR)	9 (6–14)	7 (7–14)	0.55
Recurrent pain in last 14 days	57%	21%	<0.001
Pain associated with loose stools	71%	48%	<0.001
Reported bloating	57%	17%	<0.001
Reported urgency	52%	30%	0.08
Stools often loose or watery	59%	13%	<0.001

HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, HADS–Depression subscale; PHQ-12 SS, Patient Health Questionaire-12 Somatic Symptom Scale.

Table 2 Features of postinfective bowel dysfunction 3 months after Campylobacter infection comparing cases versus controls

	Cases (n=22)	Controls (n=77)	RR (95% CI)	P value
Stools often loose or watery?	12 (55%)	12 (16%)	3.8 (1.9 to 7.4)	<0.001
Stools often hard or lumpy?	2 (9%)	15 (19%)	0.5 (0.1 to 1.5)	0.347
<3 bowel movements per week	0 (0%)	3 (4%)	0 (0 to 2.6)	>0.999
>3 bowel movements per day	6 (27%)	10 (13%)	1.9 (0.9 to 3.9)	0.185
Presence of mucus	1 (5%)	1 (1%)	2.3 (0.4 to 5.1)	0.397
Straining on defecation	4 (18%)	9 (12%)	1.5 (0.6 to 3.2)	0.477
Sensation of incomplete evacuation	10 (45%)	18 (23%)	2.1 (1.0 to 4.2)	0.06
Sensation of abdominal bloating	13 (59%)	14 (18%)	3.9 (1.9 to 7.9)	<0.001
Abdominal swelling	8 (36%)	6 (8%)	3.5 (1.7 to, 6.4)	0.002
Urgency	11 (50%)	18 (23%)	2.4 (1.2 to 4.8)	0.031
IBS by Rome III criteria?	10 (45%)	0	N/A	N/A

IBS, irritable bowel syndrome; N/A, not available.

falce discovery rate (FDR) approach, and FDR-adjusted p values (q-values) below 0.05 were considered to be significant.

RESULTS

Clinical study

There were 22 of the 99 subjects who completed the trial, who reported that their bowels had not returned to normal after the infection (cases) and 77 subjects whose bowels had normalised (controls). As table 1 shows, cases were significantly more likely to be younger, female and scored significantly higher on the assessment of somatisation. The main features recorded in the PHQ-12 SS distinguishing cases from controls were trouble sleeping, headaches, back and limb pain and lethargy (figure 2A). The main features of the BD included more bloating, more frequent episodes of pain associated with loose stools, more urgency and stools being more often loose or watery (see table 1).

Characterising PI-BD

Cases were characterised by significantly looser stools 3 months after infection (figure 2B and table 2). Stool water content of cases was significantly greater than controls (cases, n=14, mean (SD) 77.95 (6.70)%; controls, n=23, mean (SD) 71.97 (7.83)%, Fisher's exact test p=0.04, figure 2C). In addition, cases more often reported a sensation of urgency and bloating, and visible swelling of the abdomen (table 2). Rome III criteria for IBS were fulfilled in 10 (45%) cases who were very similar to the remaining 12 that did not meet the criteria (PI-BD) with no significant difference in age, anxiety, depression nor PHQ-12 SS.

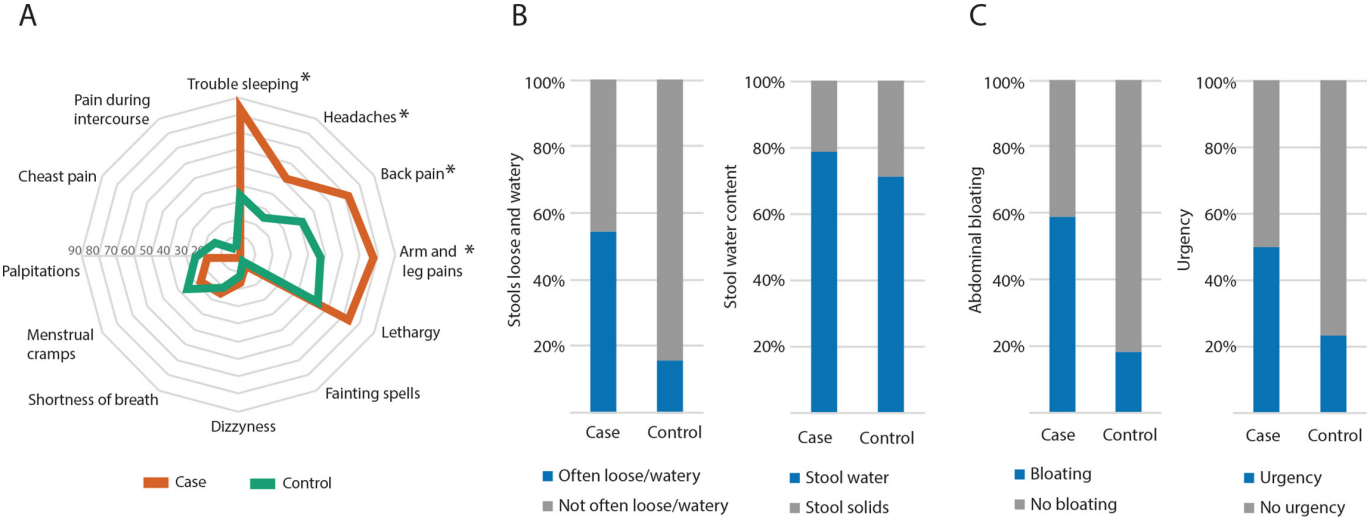

Figure 2 Differences in patients' symptoms 3 months after gastroenteritis (A) average PHQ-12S scores for cases and controls, showing the increased prevalence of trouble sleeping (p<0.0001), headaches (p=0.034), back pain (p=0.015) and limb pain (p=0.0248) in cases. Statistical significanse indicated with asterisk. (B) Proportion of loose and watery stools and water content. Cases were significantly more likely to report loose/watery stools which was confirmed with the significant difference in stool water content (p=0.04). (C) GI-symptoms. The cases also reported significantly more sensations of bloating (p<0.001) and urgency (p<0.001). GI, gastrointestinal; PHQ-12S, Patient Health Questionnaire-12 Somatic.

In addition, markers of severity of gastroenteritis did not differ significantly between PI-BD or PI-IBS, including fever, blood in stool, vomiting nor antibiotic consumption (see online supplemental table S1).

Markers of gastroenteritis severity

We found that cases were significantly more likely to report a fever during gastroenteritis (82% cases and 55% controls, p=0.02) but other markers of severity such as blood in stool, vomiting, days off work or weight loss were not significantly different between cases and controls (see online supplemental table S2).

Effect of antibiotics and concomitant medication on disease recovery

There was no significant difference in the proportion of cases versus controls who received antibiotic prescription (41% and 32%, respectively, Fisher's exact test p=0.45). Patients who received antibiotics did not appear to have any worse symptoms during the initial illness and had no clinical features significantly different from those who did not (see online supplemental table S3), however, they were significantly more likely to attend their general practitioner (GP) more than once for this illness (50% vs 28%, Fisher's exact test p=0.05). Most of our patients were healthy and taking no medication, which can of course affect the microbiota. A small number of both patients and controls took a range of medications with no consistent difference between the groups (online supplemental table S4).

Dietary habits

A subset of the subjects' (19 cases and 31 controls) dietary habits as well as faecal SCFA concentrations (14 cases and 23 controls) were assessed from visits 2 and 3. There were no significant differences in any of the nutrition components or faecal SCFAs between cases and controls or between either of the time points (online supplemental table S5).

Microbiota study

The demographics and disease severity of both the cases and controls in the mechanistic study did not differ significantly from those of the larger cohort (see online supplemental table S6 and S7, respectively).

Microbiota composition in samples collected less than 40 days after gastroenteritis is impacted by infection

The largest influence on the microbiota composition was the time since the initial infection, with a gradual recovery over the 12 weeks of study. The early samples, collected less than 40 days after reported infection, were significantly different from the later samples (MANOVA, p=0.001, figure 3A). The differences in microbiota recovery are characterised in online supplemental table S8-S10. In addition, there were significant differences in microbiota recovery in cases as compared with the controls (MANOVA, p=0.045, figure 3B,C). These significant changes were due to increased levels of the genera *Collinsella* (mean relative abundance 10.7% in cases vs 4.31% in controls, negative binomial generalised linear model q≤0.001) and *Eggerthella* (1.82% in cases vs 0.18% in controls, negative binomial generalised linear model, q=0.06, (online supplemental table S8). In addition, there was a significant decrease among cases in many taxa belonging to Firmicutes phyla, these included reduced levels of genera *Faecalibacterium* (6.06% in cases vs 8.45% in controls, negative binomial generalised linear model, q<0.001), *Enterococcus* (0.05% in cases vs 0.39% in controls, negative binomial generalised linear model, q=0.003) and taxa from the *Ruminococcaceae* family (11.66% in cases vs 18.22% in controls, negative binomial generalised linear model, q<0.001) (online supplemental table S8).

Microbiota recovery

We aimed to focus on the difference in microbiota recovery between cases and controls and concentrated on the late samples collected more than 60 days after the reported infection when

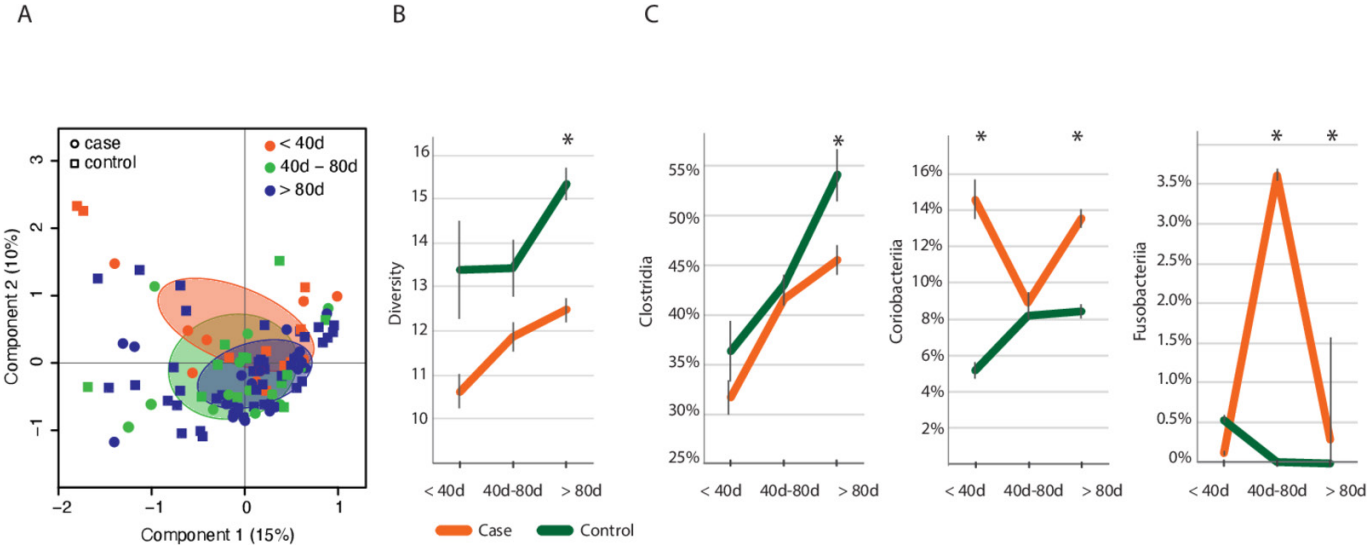

Figure 3 Microbiota recovery after infection in cases and controls. (A) PCoA plot with Bray-Curtis dissimilarity from all subjects. The largest variation in microbiota composition is due to time since infection, samples obtained early after infection being significantly different from the later ones (MANOVA multivariate analysis of variance, p=0.001). The coloured circles represent 50% of the data. (B) Inverse Simpson diversity. Microbial recovery during the follow-up period was different between cases and controls. The inverse Simpson diversity shows that cases fail to recover to normal levels in samples collected more than 80 days after infection. (C) Proportion of total of Clostridia, Coriobacteriia and Fusobacteria. There were also significant class level differences including lower clostridia, but higher *Coriobacteriia* and *Fusobacteria* (for details, see online supplemental table S8-S10). SE of mean is shown as whiskers and statistically significant difference (p<0.05) is shown with asterisk. PCoA, principal co-ordinate analysis.

Table 3 The significantly different taxa between cases and controls in samples collected more than 60 days after reported infection

Phylum	Class	Order	Family	Genus	Cases (n=18)	Controls (n=48)	Fold change
Actinobacteria	Actinobacteria	Actinomycetales	*Actinomycetaceae*	*Actinomyces*	0.16%	0.06%	2.75
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*		13.61%	9.23%	1.47
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Eggerthella*	2.59%	0.79%	3.28
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Gordonibacter*	0.35%	0.05%	6.38
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Butyricimonas*	0.03%	0.10%	0.27
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Porphyromonas*	0.02%	0.12%	0.12
Firmicutes					55.57%	64.85%	0.86
Firmicutes	Clostridia	Clostridiales			44.60%	53.56%	0.83
Firmicutes	Clostridia	Clostridiales	*Christensenellaceae*	*Christensenella*	0.35%	0.71%	0.50
Firmicutes	Clostridia	Clostridiales	*FamilyXIIIIncertaeSedis*		0.07%	0.19%	0.38
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*		17.76%	23.69%	0.75
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*	0.13%	0.28%	0.46
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Kandleria*	0.15%	0.10%	1.51
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00%	0.21%	0.01
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Phascolarctobacterium*	0.06%	0.41%	0.16
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Dialister*	1.84%	1.16%	1.58
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Veillonella*	0.09%	0.27%	0.34
Fusobacteria					0.26%	0.01%	19.32
Fusobacteria	Fusobacteriia	Fusobacteriales	*Fusobacteriaceae*	*Fusobacterium*	0.26%	0.01%	19.32
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*	*Burkholderia*	0.04%	0.00%	16.42
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	*Desulfovibrionaceae*	*Desulfovibrio*	0.02%	0.22%	0.11
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Klebsiella*	0.73%	0.02%	35.42
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*	*Haemophilus*	0.02%	0.08%	0.22

The mean relative abundance of each taxa is shown along with the fold change in cases versus controls.

we had the most samples since those who had taken antibiotics were no longer excluded. In these samples, alpha diversity (mean 12.1 in cases vs 15.8 in controls, ANOVA, p=0.015) and richness (mean 132.1 in cases vs 149.2 in controls, ANOVA, p=0.017, online supplemental figure S1) were significantly decreased in cases when compared with controls. Furthermore, the abundance of several taxa were significantly different between cases and controls in samples collected >60 days after reported infection (table 3). There was a significant decrease in the abundance of bacteria from the phylum Firmicutes, especially taxa from the order Clostridiales, which were reduced by 20.1% when compared with controls. More specifically, taxa belonging to Clostridiales such as *Ruminococcaceae* and *Christensenella* were both significantly reduced in cases. Moreover, there were two genera of Coriobacteria (Eggerthella and Goronibacter) that were more abundant in cases and the abundance of the family Coriobacteriaceae was increased by 32.2% in those with persistent BD in samples collected more than 60 days after reported infection. In addition, Fusobacteria and several taxa from the phylum Proteobacteria were increased in cases, these included a 35.4-fold increase of *Klebsiella* (a member of the Gammaproteobacteria class).

Associations between microbiota and dietary components, SCFA and stool water content

Although the cases and controls did not differ in their dietary habits (54 records in total) or SCFA concentrations (52 records in total) we found several associations with their microbiota profiles. There were 38 significant associations computed with linear models between the microbiota composition and measured SCFAs (online supplemental table S11) all values were also supported with significant spearman correlation. These included the positive association between butyric acid and the genus *Faecalibacterium* (linear mixed effects (log), q=0.09, r=0.384) and negative association between the total SCFA concentration and Gammaproteobacteria (generalised linear mixed models, q=0.01, r=−0.36). In addition, there were 23 associations to food components. Most strikingly, there was a strong negative association between levels of Gammaproteobacteria and the consumption of fibre (generalised linear mixed models, q=0.03, r=−0.46, figure 4), non-starch polysaccharides (generalised linear mixed models, q=0.05, r=−0.47) and starch (generalised

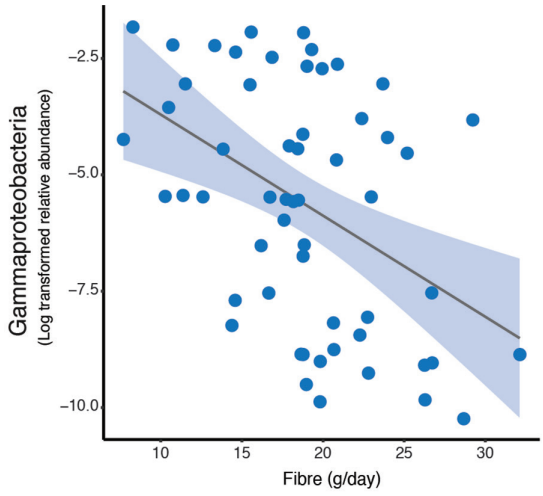

Figure 4 Association between fibre consumption and gammaproteobacterial abundance. The association was statistically significant (q=0.032), where low consumption of fibre was associated with high Gammaprotebacteria abundance. Light area indicates SE of mean.

linear mixed models, q=0.003, r=−0.43, online supplemental Table S12). Increased stool water content was associated with increased levels of the class betaproteobacteria (generalised linear mixed models, q=0.03, r=−0.23).

DISCUSSION

We confirmed previous findings that PI-BD followed by Campylobacter infection is characterised by loose stools, bloating and urgency suggesting faster overall transit.[12 16 24 25] What determines this change in function is unclear, but we now report that the microbiota recovery from gastroenteritis was slower and less complete in PI-BD cases than controls. A key feature which could be relevant to the ongoing new symptoms includes a significantly lower diversity, which we found in the early samples. This was significantly greater in cases as compared with controls and this persisted more than 60 days after the reported infection, regardless of antibiotic use. This is likely to be due to inflammation since similar loss of diversity has been reported in association with Crohn's disease[26 27] and after norovirus infection,[28] which, as we found, were also associated with increased Proteobacteria. In our study, cases did not differ from controls in antibiotic use nor disease severity except a much greater proportion (94% vs 55%) reported fever. The changes in microbiota are likely therefore to reflect the combined effect of the resilience of the original microbiota together with the patient's inflammatory response to *C. jejuni*. This depletes normal commensal bacteria and, by reducing colonisation resistance, allows the pathogen to proliferate.[29]

The adult gut microbiome characteristically exists in a steady state requiring a major disturbance, such as a bout of gastroenteritis, to alter that state permanently. Indicative of such a shift in the cases of this cohort is the large and persistent changes in the major bacterial classes including the decreased levels of Clostridia, a taxon often associated with health benefits such as SCFA production. We found that the decrease in Clostridia was mirrored by the increase in classes such as Gammaproteobacteria in the cases as compared with controls more than 60 days after infection. Interestingly the levels of Gammaproteobacteria were inversely associated with total SCFAs and more specifically butyrate and propionate acids. In addition, the patient's consumption of fibre, non-digestible polysaccharides and starch were negatively associated with Gammaprotebacteria abundance. There is substantial evidence that the health benefits of high fibre consumption are mediated in part via increased SCFA production which decreases pH in the colon, inhibiting the growth of Gammaproteobacteria.[30–32] Taken together, this suggests high fibre diets could contribute to correcting the microbiota disturbance and preventing PI-BD, something which should be further evaluated in randomised controlled clinical trials.

We showed here cases have a significant reduction in microbial diversity and the total Firmicutes, especially taxa from *Clostridiales* and *Ruminococcaceae* groups. This may reflect continuing disturbance of transit as reflected by increased stool water content and reporting loose or watery stools. This is in line with previous findings where, even in healthy subjects, soft stools were associated with reduced diversity.[33] Most individuals with firmer stools in that study had the *Ruminococcaeae-Bacteroides* enterotype showing how different consistency favours different species. Both fast transit and mucosal inflammation disturb the anaerobicity of the colonic environment, which depletes the strict anaerobes and allows facultative anaerobes and those with rapid replication such as Gammaproteobacteria and Fusobacteria, to proliferate and occupy the vacant ecological niche. Similar reductions in Firmicutes have been recently reported in children from Peru who were hospitalised with gastroenteritis, particularly those with bacterial infections like Campylobacter, Shigella and Salmonella.[34] Similarly, the persistent reduction of Firmicutes and increased Proteobacteria seen in IBD is thought to represent increased availability of small molecules created by the inflammatory process such as nitric oxide and reactive oxygen species that can act as electron acceptors for facultative anaerobes like Proteobaceria.[35]

Several members of the *Coriobacteriea* family were increased in our PI-BD cases very early after the infection and this increased abundance persisted throughout the study. Previous studies have also associated this family with IBS.[36–38] Vich Vila *et al* who studied a cohort of 412 patients with IBS with shotgun metagenomics showed that IBS patients had increased levels of Coriobacteria, especially the genus *Eggerthella*. This was complemented with the decreased abundance of several important clostridial species including *Ruminococcaceae,*[37] a pattern also detected in our cases. A similarly increased abundance of *Coriobacteriaceae, Proteobacteria* and *Fusobacteria* has been reported after Roux-en-Y surgery for obesity.[39 40] The common aspect shared with previous findings and our PI-BD patients may be faster transit through the gut, which alters the colonic milieu in multiple ways including reducing secondary bile acids, raising pH and reducing SCFAs.

Although *Fusobacterium* accounts for only a small percentage of total bacteria it was markedly higher in our cases through-out the study. *Fusobacterium* has also been noted to be part of a characteristic cluster of organisms that bloom immediately after *V. cholera* infection.[6] The pattern of low Firmicutes and increased *Fusobacterium* is of special interest since in stressed maternally separated rats the same pattern is seen and the severity of hypersensitivity to rectal distension in maternally deprived rats was correlated with *Fusobacterium* numbers.[41] Furthermore, when gavaged into rats, *Fusobacterium* induces visceral hypersensitivity.[42]

Previous studies suggested that the risk of PI-BD increased proportionate to the severity of the initial insult.[16] We found that fever was an important risk factor in developing PI-BD, possibly a marker of severity reflecting the increased permeability due to *C. jejuni* infection[43] allowing systemic access of pyrogens such as lipopolysaccharide. Our findings differ from a recent meta-analysis where receiving antibiotics was deemed a risk factor for developing PI-IBS.[1] We did, however, find those receiving antibiotics were more likely to make more than one visit to their GP despite having similar markers of illness severity so it may reflect underlying differences in healthcare seeking behaviour rather than a direct effect of antibiotics. This is supported by our finding that cases had a significantly elevated PHQ12-SS, confirming other studies which have indicated that adverse psychological features such as neuroticism,[44] depression[12] and multiple non-gastrointestinal somatic symptoms[2] increase the risk of postinfective IBS. As the recent meta-analysis[45] reported females have an increased relative risk compared with males of developing PI-IBS, mean (95% CI) 2.2 (1.6 to 3.1). Relative risk in our study at 4.2 was higher despite an equal number of males and females taking part but why is unclear and gender did not appear to affect the microbiota.

Only a small proportion of the total 1286 infected patients chose to take part which raises the question of bias. However, the proportion of subjects developing PI-BD, 22% was in fact very close to the 25% reported in our less demanding survey previously reported in which response rate was much higher

at 72%.[16] This suggests that the severity of bowel disturbance is not a major factor in determining participation, but a multitude of other factors like altruism, proximity to study site and ability to take time of work. Those with PI-BD did show greater somatisation which has been found in other studies[1 2] but the underlying mechanisms are unclear. By choosing those whose bowels returned to normal as controls we aimed to control for the many factors which influence both getting infectious gastroenteritis, attending a doctor and sending a stool sample to the Public Health laboratory which include age, gender, severity and most importantly the GPs beliefs, which vary widely.[46] The samples at 3 months of those who report bowel function back to normal would seem to be the best estimate of what is normal for the controls. Sampling was also limited by administrative obstacles which mean we could not get samples as early as we would have wished when the changes might have been more substantial, however, since our main focus is the long-term effects this is perhaps not such a limitation. Our attempt to avoid the effects of antibiotics by analysing samples taken at least 60 days after antibiotic consumption represents a compromise since excluding all 9/22 cases who took antibiotics would have seriously underpowered our study.

An important limitation of a descriptive study such as ours is that it does not allow one to distinguish cause from effect. An alternative interpretation of the lower diversity in cases is that those with lower initial diversity are less resilient and hence predisposed to a more severe infection and disturbance of gut function. Interestingly in a prospective study of Campylobacter infection among abattoir workers, a pre-existing higher abundance of *Bacteroides* and *E. coli* increased the risk of developing Campylobacter enteritis[47] suggesting that this profile leads to lower colonisation resistance. Our study adds to the existing information and invites further studies both to confirm the findings but also to include interventions such as high fibre/prebiotics or drugs to slow transit that might normalise the microbiota and improve symptoms.

Correction notice This article has been corrected since it published Online First. Figure 3 has been replaced. The open access licence has also been updated to CC BY.

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Supplementary Tables

Supplementary Table S1: Demographics and markers of disease severity in cases, comparing those who fulfilled Rome III criteria for post-infectious irritable bowel syndrome (PI-IBS) and those who did not (Post-infectious bowel dysfunction; PI-BD)

	PI-IBS	PI-BD	p-value
Number of cases	10	12	
Age, median (IQR)	58 (45-69)	54 (42-63)	0.57
HADS-A, mean (SD)	8 (6)	6 (3)	0.36
HADS-D, median (IQR)	4 (3-9)	2 (1-8)	0.22
PHQ-12 SS, mean (SD)	5 (2)	4 (2)	0.82
Fever, *n* (%)	9 (90%)	9 (75%)	0.59
Blood in stool, *n* (%)	1 (10%)	3 (25%)	0.59
Vomiting, *n* (%)	3 (30%)	2 (17%)	0.62
Antibiotics taken, *n* (%)	3 (30%)	6 (50%)	0.41

HADS-A, Hospital Anxiety and Depression Scale – Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale – Depression subscale; PHQ-12 SS, Patient Health Questionaire-12 Somatic Symptom Scale.

Supplemental Table S2: Markers of initial illness severity in cases and controls

	Cases (n=22)	Controls (n=77)	RR (95% CI)	*p* value
Fever, *n* (%)	18 (82%)	42 (55%)	2.9 (1.2, 7.9	**0.02**
Blood in stool, *n* (%)	4 (18%)	21 (27%)	0.7 (0.2, 1.6)	0.58
Vomiting, *n* (%)	5 (23%)	16 (21%)	1.1 (0.4, 2.4)	>0.99
Antibiotics taken, *n* (%)	9 (41%)	25 (32%)	1.3 (0.6, 2.7)	0.46
>7 days unable to do normal activity, *n* (%)	10 (45%)	40 (52%)	0.8 (0.4, 1.7)	0.64
weight loss (kg), median (IQR)	3.2 (1.8-4.5)	3.2 (2.5-4.5)	-	0.48

Supplementary Table S3: Demographics and psychological scores of those who took antibiotics and those who did not, and the effect of consumption on disease recovery

	Antibiotics (n=34)	**No antibiotics (n=65)**	***p*-value**	**RR (95% CI)**
Age, mean (SD)	59 (12)	58 (16)	0.84	-
Female, *n*(%)	18 (53%)	32 (49%)	0.83	1.1 (0.6, 1.9)
HADS-A, median (IQR)	6 (4-9)	5 (3-8)	0.36	-
HADS-D, median (IQR)	3 (2-6)	3 (1-6)	0.46	-
PHQ-12, median (IQR)	3 (1-5)	3 (1-5)	0.57	-
Fever, *n* (%)	23 (68%)	37 (57%)	0.39	1.4 (0.8, 2.5)
Blood in stool, *n* (%)	12 (35%)	13 (20%)	0.14	1.6 (0.9, 2.7)
Vomiting, *n* (%)	5 (15%)	16 (25%)	0.31	0.6 (0.3, 1.3)
>1 GP attendance for this illness, *n* (%)	17 (50%)	18 (28%)	**0.05**	1.8 (1.0, 3.1)
Attended hospital emergency rooms for this illness, *n* (%)	3 (12%)	14 (22%)	0.28	0.6 (0.2, 1.3)
Hospitalised for this illness, *n* (%)	3 (9%)	8 (12%)	0.74	0.8 (0.3, 1.7)
Weight loss (kg), median (IQR)	3.2 (2.3-3.8)	3.2 (2.6-4.5)	0.34	-
Days unable to do normal activity, median (IQR)	10 (4-16)	8 (3-13)	0.35	-

HADS-A, Hospital Anxiety and Depression Scale – Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale – Depression subscale; PHQ-12 SS, Patient Health Questionaire-12 Somatic Symptom Scale.

Supplementary Table S4: Concomitant medication

	Cases (n=22)	Controls (n=77)
Atorvastatin/ simvastatin	2	3
Ramipril	1	3
Metformin	0	2
Lanzoprazole/ omeprazole	0	3
Amlodipine	0	2
Bisoprolol/ atenolol	0	3
Glicazide	0	1
Fluoxitine / Sertraline	0	2

Supplementary Table S5: Dietary and short chain fatty acid (SCFA) amounts per study group and time point. There were no statistical differences (p>0.05) between any of the groups or time points. Data are given as mean (SD).

	Visit 2		Visit 3	
	Case	Control	Case	Control
Energy (kcal/day)	2032.90 (772.75)	2103.54 (823.43)	1907.11 (596.5)	1690.56 (246.86)
Protein (g/day)	72.88 (20.73)	88.31 (37.26)	68.18 (25.3)	71.67 (16.97)
Fat (g/day)	83.83 (32.36)	87.13 (44)	72.769 (30.78)	66.25 (16.29)
CHOm (g/day)	229.54 (85.89)	234.8 (84.51)	226.13 (65.43)	198.36 (41.16)
NSP (g/day)	13.39 (4.93)	14.61 (6.27)	11.68 (3.67)	11.52 (2.89)
Fibre (g/day)	19.05 (6.77)	21.48 (8.31)	16.81 (5.13)	17.44 (4.58)
total SCFA (µmol/g)	44.59 (13.12)	43.27 (11.93)	41.53 (7.47)	41.46 (12.52)
Acetic (µmol/g)	28.01 (6.26)	25.87 (4.87)	25.43 (3.73)	24.77 (5.02)
Propanoic (µmol/g)	6.69 (4.20)	7.14 (3.47)	6.72 (3.20)	6.79 (5.10)
Isobutyric (µmol/g)	0.86 (0.36)	1.23 (0.59)	0.98 (0.31)	1.19 (0.44)
Butyric (µmol/g)	6.95 (4.39)	6.50 (4.22)	6.13 (2.84)	6.00 (2.84)
Isovaleric (µmol/g)	1.03 (0.53)	1.49 (0.81)	1.14 (0.46)	1.52 (0.63)
Valeric (µmol/g)	1.06 (1.17)	1.04 (0.61)	1.13 (0.77)	1.18 (0.75)

Supplemental Table S6: Comparison of demographics, psychological scores and markers of disease severity between cases in the clinical and mechanistic studies

	Clinical study cases	Mechanistic study cases	p value
Number of subjects	22	18	-
Age, median (IQR)	57 (41-64)	56 (39-62)	0.96
Female, *n* (%)	17 (77%)	14 (78%)	0.99
HADS-A, median (IQR)	5 (4-10)	8 (4-10)	0.78
HADS-D, median (IQR)	4 (1-6)	4 (1-7)	0.99
PHQ-12 SS, mean (SD)	5 (3-6)	5 (2-6)	0.85
Fever, *n* (%)	18 (82%)	17 (94%)	0.52
Blood in stool, *n* (%)	4 (18%)	4 (22%)	0.84
Vomiting, *n* (%)	5 (23%)	5 (28%)	0.78
Antibiotics taken, *n* (%)	9 (41%)	5 (28%)	0.70

Supplementary Table 7: Comparison of demographics, psychological scores and markers of disease severity between controls in the clinical and mechanistic studies

	Clinical study controls	Mechanistic study controls	p value
Number of subjects	77	48	-
Age, median (IQR)	62 (48-71)	63 (48-71)	0.69
Female, *n* (%)	33 (43%)	20 (42%)	0.91
HADS-A, median (IQR)	5 (3-7)	5 (3-7)	0.95
HADS-D, median (IQR)	3 (1-5)	3 (1-6)	0.67
PHQ-12 SS, mean (SD)	2 (1-4)	2 (1-4)	0.73
Fever, *n* (%)	42 (55%)	24 (50%)	0.67
Blood in stool, *n* (%)	21 (27%)	9 (19%)	0.42
Vomiting, *n* (%)	16 (21%)	12 (25%)	0.70
Antibiotics taken, *n* (%)	25 (32%)	14 (29%)	0.76

Supplementary Table S8: Significant microbiota differences between cases and controls in samples collected less than 40 days after infection. All samples effected by the antibiotic use were excluded from the analysis. The mean relative abundance of each taxa is shown along with the fold change in cases versus controls.

Phylum	Class	Order	Family	Genus	Case	Control	Fold change
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*		13.83 %	5.25 %	0.38
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Collinsella*	10.70 %	4.31 %	0.40
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Eggerthella*	1.82 %	0.18 %	0.10
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Butyricimonas*	0.01 %	0.13 %	11.85
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Porphyromonas*	0.00 %	0.24 %	62.19
Bacteroidetes	Bacteroidia	Bacteroidales	*Prevotellaceae*	*Paraprevotella*	0.06 %	0.41 %	7.48
Firmicutes	Bacilli	Lactobacillales	*Enterococcaceae*	*Enterococcus*	0.05 %	0.39 %	8.27
Firmicutes	Clostridia	Clostridiales	*Lachnospiraceae*	*Anaerosporobacter*	0.16 %	0.61 %	3.71
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*		11.66 %	18.22 %	1.56
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Faecalibacterium*	6.06 %	8.45 %	1.39
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00 %	0.11 %	34.95
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Phascolarctobacterium*	0.29 %	0.25 %	0.85
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Megamonas*	0.00 %	0.69 %	579.67
Proteobacteria	Betaproteobacteria				0.48 %	1.97 %	4.12
Proteobacteria	Betaproteobacteria	Burkholderiales			0.45 %	1.80 %	4.03
Proteobacteria	Betaproteobacteria	Burkholderiales	*Alcaligenaceae*	*Sutterella*	0.40 %	1.77 %	4.41
Proteobacteria	Betaproteobacteria	Neisseriales	*Neisseriaceae*	*Neisseria*	0.03 %	0.13 %	4.12
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Klebsiella*	0.00 %	0.26 %	146.38

Supplementary Table 8: Significant microbiota differences between cases and controls in samples collected between 40 to 80 days after infection. All samples effected by the antibiotic use are excluded from the analysis. The mean relative abundance of each taxa is shown along with the fold change in cases versus controls

Phylum	Class	Order	Family	Genus	Case	Control	Fold change
Actinobacteria	Actinobacteria	Micrococcales	*Micrococcaceae*		0.03 %	0.27 %	8.54
Actinobacteria	Actinobacteria	Micrococcales	*Micrococcaceae*	*Micrococcus*	0.00 %	0.01 %	26.96
Actinobacteria	Actinobacteria	Micrococcales	*Micrococcaceae*	*Rothia*	0.03 %	0.25 %	8.17
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Olsenella*	0.00 %	0.14 %	Inf
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Barnesiella*	0.18 %	0.58 %	3.13
Firmicutes	Clostridia	Clostridiales	*Peptostreptococcaceae*	*Peptostreptococcus*	0.00 %	0.00 %	1.98
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Coprobacillus*	0.00 %	0.01 %	7.59
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Erysipelothrix*	0.00 %	0.01 %	2.02
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00 %	0.02 %	7.43
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*		0.40 %	0.94 %	2.36
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Phascolarctobacterium*	0.19 %	0.52 %	2.80
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Succiniclasticum*	0.00 %	0.08 %	38.37
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Megamonas*	2.58 %	0.07 %	0.03
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Megasphaera*	0.00 %	0.72 %	593.50
Fusobacteria	Fusobacteriia	Fusobacteriales	*Fusobacteriaceae*	*Fusobacterium*	2.24 %	0.02 %	0.01
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*		0.03 %	0.00 %	0.06
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*	*Burkholderia*	0.03 %	0.00 %	0.06
Proteobacteria	Epsilonproteobacteria	Campylobacterales	*Campylobacteraceae*	*Campylobacter*	0.02 %	0.23 %	13.99
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*	*Haemophilus*	0.06 %	0.59 %	10.31

Supplementary Table 9: Significant microbiota differences between cases and controls in samples collected more than 80 days after infection. The mean relative abundance of each taxa is shown along with the fold change in cases versus controls

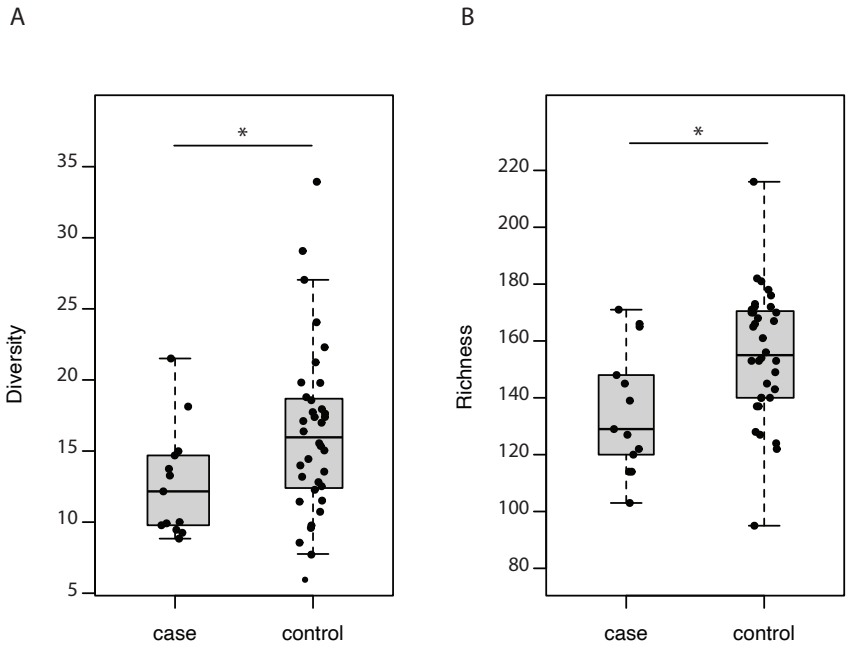
Phylum	Class	Order	Family	Genus	Case	Control	Fold change
Actinobacteria	Actinobacteria	Actinomycetales	*Actinomycetaceae*		0.11 %	0.05 %	0.45
Actinobacteria	Actinobacteria	Actinomycetales	*Actinomycetaceae*	*Actinomyces*	0.11 %	0.05 %	0.45
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*		13.56 %	8.71 %	0.64
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Eggerthella*	2.11 %	0.83 %	0.39
Firmicutes					56.67 %	66.47 %	1.17
Firmicutes	Bacilli	Lactobacillales	*Streptococcaceae*	*Lactococcus*	0.03 %	0.01 %	0.25
Firmicutes	Clostridia	Clostridiales			45.57 %	55.23 %	1.21
Firmicutes	Clostridia	Clostridiales	*FamilyXIIIIncertaeSedis*	*IncertaeSedis*	0.08 %	0.15 %	1.87
Firmicutes	Clostridia	Clostridiales	*FamilyXIIncertaeSedis*	*Peptoniphilus*	0.00 %	0.01 %	12.66
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*	0.10 %	0.25 %	2.49
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00 %	0.13 %	64.42
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Succiniclasticum*	0.08 %	0.25 %	3.31
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Dialister*	1.97 %	1.25 %	0.64
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Veillonella*	0.10 %	0.25 %	2.53
Fusobacteria	Fusobacteriia	Fusobacteriales	*Fusobacteriaceae*	*Fusobacterium*	0.12 %	0.01 %	0.08
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*	*Burkholderia*	0.03 %	0.00 %	0.11
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	*Desulfovibrionaceae*	*Desulfovibrio*	0.02 %	0.14 %	8.67
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*		0.02 %	0.06 %	2.85
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*	*Haemophilus*	0.02 %	0.06 %	2.86

Supplementary Table S11: Significant associations between bacterial taxa and short chain fatty acids. The dietary information was collected from patients visits 2 and 3. Positive association indicated with + and orange, negative association indicated with – and blue.

Phylum	Class	Order	Family	Genus	Total SCFA	Isobutyric	Butyric	Propanoic	Acetic	Valeric	Isovaleric
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Atopobium*					-		
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Collinsella*							+
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Gordonibacter*		+					+
Bacteroidetes	Bacteroidia	Bacteroidales	*Prevotellaceae*						+		
Bacteroidetes	Bacteroidia	Bacteroidales	*Prevotellaceae*	*Paraprevotella*					+	+	
Bacteroidetes	Bacteroidia	Bacteroidales	*Rikenellaceae*	*Alistipes*	-				-		
Firmicutes	Bacilli	Lactobacillales	*Enterococcaceae*		-		-	-			
Firmicutes	Bacilli	Lactobacillales	*Enterococcaceae*	*Enterococcus*	-		-	-			
Firmicutes	Clostridia				-			-	-		
Firmicutes	Clostridia	Clostridiales	*Christensenellaceae*							+	
Firmicutes	Clostridia	Clostridiales	*Christensenellaceae*	*Christensenella*						+	
Firmicutes	Clostridia	Clostridiales	*Clostridiaceae*						-		
Firmicutes	Clostridia	Clostridiales	*Clostridiaceae*	*Clostridium*					-		
Firmicutes	Clostridia	Clostridiales	*FamilyXIIncertaeSedis*		-				-		
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*					-		
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Faecalibacterium*			+				
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Flavonifractor*				-			
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Catenibacterium*		+					+
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*					+		
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Dialister*	+	+		+			
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Mitsuokella*				-			
Proteobacteria	Epsilonproteobacteria								-		
Proteobacteria	Gammaproteobacteria				-		-	-			

Supplementary table S12: Significant associations between bacterial taxa and dietary components. The dietary information was collected from patients visits 2 and 3. Positive association indicated with + and orange, negative association indicated with – and blue.

Phylum	Class	Order	Family	Genus	CHO	Fat	Fibre	NPS	Starch
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Coriobacterium*	+				
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Olsenella*	+				
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Paraeggerthella*	-				+
Firmicutes	Bacilli	Lactobacillales	*Streptococcaceae*					-	-
Firmicutes	Bacilli	Lactobacillales	*Streptococcaceae*	*Streptococcus*				-	-
Firmicutes	Clostridia	Clostridiales	*FamilyXIIIIncertaeSedis*		-			-	
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*	-				
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*IncertaeSedis*		-			
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*				-	-	
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*		-				
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Succiniclasticum*	-	-			
Proteobacteria	Betaproteobacteria					-			
Proteobacteria	Gammaproteobacteria						-	-	-
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*					-	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Klebsiella*			-		


A
B
*
Diversity
35
30
25
20
15
10
5
case
control
*
Richness
220
200
180
160
140
120
100
80
case
control

Supplementary methods

The study population

This was a single centre cohort study of patients whose stool sample tested positive for Campylobacter spp. The main clinical study included all 155 eligible subjects who provided clinical details of their illness, psychological parameters, and bowel function. These factors were tested for their association with subsequent post-infective bowel dysfunction (PI-BD). We nested within the main study a detailed mechanistic study of the microbiota in those who provided adequate stool samples. Originally, we had planned to compare just 20 in each group but advances in technology and reduction in costs allowed us to expand our controls and we were able to compare 18 cases with 45 controls, chosen because they had provided the most complete set of stool samples.
The study was approved by the Nottingham Research Ethics Committee on 29th August S (13/EM/0310) and started 1st January 2014. The study was prospectively registered on clinicaltrials.gov (NCT02040922). Although the endpoints were not changed, we initially aimed to recruit 450 to provide adequate power to assess at the impact of antibiotic use during *Campylobacter* infection on prevalence of PI-IBS and to study in depth 20 cases. Despite the fact that we only recruited 129 by the time the funding ceased, and the study closed on Oct 2016, we were able to obtain 18 out of our planned 20 cases for detailed study, whose results are presented here along with the clinical features of 111 controls.

Participants

Inclusion / exclusion criteria
Eligible participants were aged >18, with a clinical syndrome suggestive of intestinal infection, *Campylobacter sp.* in the associated stool sample and living within the Nottingham postal code area. Patients were excluded if they: were pregnant (self-declared), had a pre-existing gastrointestinal disorder or previous resection of the gastrointestinal tract (excluding appendix and gallbladder), had a chronic condition likely to require antibiotics in the next 3 months, regularly used opioids, had used antibiotics or high dose laxatives in the 4 weeks preceding the infection, were unable to complete symptom questionnaires, or were, in the opinion of the investigator, unable to comply with the protocol.

Patient identification
Patients were identified from a stool culture positive for Campylobacter in the Public Health England Laboratory in Nottingham. Data protection laws prevented direct contact so every 2 weeks a batch of letters were sent to patients who had previously been informed of their positive stool culture, inviting them to contact the research team. We hoped to get a sample as early as possible in the illness and to compare this with a sample taken around 6 weeks and 12 weeks from the initial infection, a time span during which our previous study indicated that most people who were going to recover would do so (10).

Patient visits
Visit 1 was arranged as soon as possible after a positive culture, but in the event, there was a considerable delay. Visit 2 was aimed at being 6 weeks after diagnosis though in some cases, owing to delay in making the first appointment this was merged with visit 1 while

Visit 3 was the end of the study 12 weeks after the initial infection. Owing to the delay before patients received the invitation letter the first visit and faecal sample was mean 46 (range 17-93) days and the final sample as mean 97(range 57-160) days from the start of symptoms.

At Visit 1 eligibility was confirmed and written informed consent obtained. Demographics were documented and symptom questionnaires (see below) were completed.

Stool samples

Patients were asked to collect stool samples for each visit, either bringing them within 2 hours of passage to the laboratory for immediate freezing at -80°C or storing at home, double bagged in their domestic freezer at -180C, before bringing in an insulated bag to the laboratory for storage at -80°C prior to analysis.

If Visit 1 occurred within 5 weeks of diagnosis, patients were asked to return for Visit 2 at 6 weeks (typically one week later) to provide a further stool sample. At Visit 3, 12 weeks after diagnosis, patients were asked to complete a questionnaire on their bowel symptoms from the past week and provide a further stool sample

Recruitment

Eligible patients were recruited through the Nottingham University Hospitals Microbiology Laboratory by the Health Protection Team with an invitation letter, information sheet and questionnaires on gastrointestinal symptoms prior to and during the infection. Despite sending out 1286 invitations, recruitment was much slower than expected and after the allotted time of 2 years the study was closed with only 155 patients recruited, of which 99 completed the study (see manuscript Figure 1).

Symptom questionnaires

At Visit 1 patients completed questionnaires documenting demographics prior to infection including prior bowel habit using the Rome III questionnaire, anxiety and depression using the Hospital Anxiety and Depression scale (HADS) [1] and somatisation using the Patient Health Questionnaire -12 Somatic Symptom Scale (PHQ-12 SS) [2]. They were also asked about features of the acute illness with markers of severity including rectal bleeding, weight loss and duration of time off normal activities. We also documented any antibiotic treatment. At each visit they reported their bowel habit during the previous week. At Visit 3, cases were defined as those responding “No” to the question “have your bowels returned to normal” and controls were those who responded “Yes”.

Lack of significant difference between cases with PI-BD and those meeting Rome III criteria

Just under half of the cases (45%) met the Rome criteria III for IBS. These subjects (PI-IBS) were very similar to the remaining 12 not meeting the criteria (PI-BD) with no significant difference in age (mean 57, SD=±14 versus 53 ±15), anxiety (8 ± 6 versus 6 ± 3), depression (median (IQR), 4(2.8-8.5) versus 2(1-7.5)) nor PHQ-12 SS (5(2) versus 4(2)). Neither did the markers of severity differ significantly between PI-BD or PI-IBS, including fever (9/10 versus 9/12), blood in stool (1/10 versus 3/12), vomiting (3/10 versus 2/12) and antibiotic consumption (3/10 versus 6/12).

Microbiota sequencing

The sequencing reads were processed using R package mare and functions ProcessReads and TaxonomicTable. Here the quality filtering, chimera detection, and taxonomic annotation functions rely on USEARCH (version 8.1.1756_i86osx32), [3]. We used only the forward reads for the taxonomical annotation. After removing the primer sequence the reads were trimmed to 180 bases, which were then used for taxonomical assignment. [4] The reads were summed to ASV and reads with less than 68 replicates in all samples were excluded as potentially incorrect and removed from further analysis. The taxonomic annotation was performed using USEARCH by mapping the reads to the SILVA 16S rRNA reference database version 115, curated to contain only sequences matching to the forward and reverse primers that were used for sequencing. No other normalization method was applied after pre-processing. The sequencing controls were assessed and since all the sequencing controls contained less than 1000 reads, the potential contaminants were not removed from the original reads due to very low impact.

Statistical analysis:

In the statistical analysis we used the GroupTest and CovariateTest functions of mare package.[5] The CovariateTest function tests for associations between the desired taxa and a continuous variable. Whereas the group test uses each taxon to test the statistical difference between the studied groupings. Both functions consider the read depth of each sample and uses that as an offset for the model. All taxonomical levels were used as relative data in the analysis from genus level taxa up until phylum level data. The pre-processed data was not transformed in any other way.

There are several statistical models that can be applied in the GroupTest and CovariateTest functions. When having multiple samples from one subject, the subject ID information is used as and will use that variable as the random factor in the model. the function first attempts to fit a zero-inflated negative binomial model using the glmmADMB package. Violation against model assumptions e.g., homogeneity of residuals may lead to meaningless p-values and potentially false conclusions. Therefore, this is considered with in the GroupTest function. If the initial model fails to produce reliable results, glm.nb function, linear models (lm), generalised least squares models (gls), or linear mixed models (lme) are fitted, depending on the situation. If all tests fail, no p-values are given. All obtained p-values are corrected for multiple testing and q-values are produced.

We tested the association between alpha diversity and read counts. The sequenced samples had a median read count of 51893 (lower hinge of 46422 and upper hinge of 57773) indicating that there is not very much difference in the read depth of the sequenced samples. We tested the spearman correlation between read counts and both microbial richness (cor = 0.19, p = 0.03) and diversity (cor = 0.084, p = 0.33). Although the p-value of the correlation between read count and richness is bellow 0.05, the actual association is weak and is due to only few samples (data not shown). We therefore did not see it necessary to adjust the richness and diversity measures with read depth.

References:

1 Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;**67**:361-70.

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3 Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;**26**:2460-1.

4 Korpela K, Blakstad EW, Moltu SJ, Strommen K, Nakstad B, Ronnestad AE*, et al.* Intestinal microbiota development and gestational age in preterm neonates. Sci Rep 2018;**8**:2453.

5 Korpela K. mare: Microbiota Analysis in R Easily. R package version 1.0. https://github.com/katrikorpela/mare DOI:10.5281/zendo.50310. 2016.

Supplementary Tables

Supplementary Table S1: Demographics and markers of disease severity in cases, comparing those who fulfilled Rome III criteria for post-infectious irritable bowel syndrome (PI-IBS) and those who did not (Post-infectious bowel dysfunction; PI-BD)

	PI-IBS	PI-BD	p-value
Number of cases	10	12	
Age, median (IQR)	58 (45-69)	54 (42-63)	0.57
HADS-A, mean (SD)	8 (6)	6 (3)	0.36
HADS-D, median (IQR)	4 (3-9)	2 (1-8)	0.22
PHQ-12 SS, mean (SD)	5 (2)	4 (2)	0.82
Fever, *n* (%)	9 (90%)	9 (75%)	0.59
Blood in stool, *n* (%)	1 (10%)	3 (25%)	0.59
Vomiting, *n* (%)	3 (30%)	2 (17%)	0.62
Antibiotics taken, *n* (%)	3 (30%)	6 (50%)	0.41

HADS-A, Hospital Anxiety and Depression Scale – Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale – Depression subscale; PHQ-12 SS, Patient Health Questionaire-12 Somatic Symptom Scale.

Supplemental Table S2: Markers of initial illness severity in cases and controls

	Cases (n=22)	Controls (n=77)	RR (95% CI)	*p* value
Fever, *n* (%)	18 (82%)	42 (55%)	2.9 (1.2, 7.9	**0.02**
Blood in stool, *n* (%)	4 (18%)	21 (27%)	0.7 (0.2, 1.6)	0.58
Vomiting, *n* (%)	5 (23%)	16 (21%)	1.1 (0.4, 2.4)	>0.99
Antibiotics taken, *n* (%)	9 (41%)	25 (32%)	1.3 (0.6, 2.7)	0.46
>7 days unable to do normal activity, *n* (%)	10 (45%)	40 (52%)	0.8 (0.4, 1.7)	0.64
weight loss (kg), median (IQR)	3.2 (1.8-4.5)	3.2 (2.5-4.5)	-	0.48

Supplementary Table S3: Demographics and psychological scores of those who took antibiotics and those who did not, and the effect of consumption on disease recovery

	Antibiotics (n=34)	**No antibiotics (n=65)**	***p*-value**	**RR (95% CI)**
Age, mean (SD)	59 (12)	58 (16)	0.84	-
Female, *n*(%)	18 (53%)	32 (49%)	0.83	1.1 (0.6, 1.9)
HADS-A, median (IQR)	6 (4-9)	5 (3-8)	0.36	-
HADS-D, median (IQR)	3 (2-6)	3 (1-6)	0.46	-
PHQ-12, median (IQR)	3 (1-5)	3 (1-5)	0.57	-
Fever, *n* (%)	23 (68%)	37 (57%)	0.39	1.4 (0.8, 2.5)
Blood in stool, *n* (%)	12 (35%)	13 (20%)	0.14	1.6 (0.9, 2.7)
Vomiting, *n* (%)	5 (15%)	16 (25%)	0.31	0.6 (0.3, 1.3)
>1 GP attendance for this illness, *n* (%)	17 (50%)	18 (28%)	**0.05**	1.8 (1.0, 3.1)
Attended hospital emergency rooms for this illness, *n* (%)	3 (12%)	14 (22%)	0.28	0.6 (0.2, 1.3)
Hospitalised for this illness, *n* (%)	3 (9%)	8 (12%)	0.74	0.8 (0.3, 1.7)
Weight loss (kg), median (IQR)	3.2 (2.3-3.8)	3.2 (2.6-4.5)	0.34	-
Days unable to do normal activity, median (IQR)	10 (4-16)	8 (3-13)	0.35	-

HADS-A, Hospital Anxiety and Depression Scale – Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale – Depression subscale; PHQ-12 SS, Patient Health Questionaire-12 Somatic Symptom Scale.

Supplementary Table S4: Concomitant medication

	Cases (n=22)	Controls (n=77)
Atorvastatin/ simvastatin	2	3
Ramipril	1	3
Metformin	0	2
Lanzoprazole/ omeprazole	0	3
Amlodipine	0	2
Bisoprolol/ atenolol	0	3
Glicazide	0	1
Fluoxitine / Sertraline	0	2

Supplementary Table S5: Dietary and short chain fatty acid (SCFA) amounts per study group and time point. There were no statistical differences (p>0.05) between any of the groups or time points. Data are given as mean (SD).

	Visit 2		Visit 3	
	Case	Control	Case	Control
Energy (kcal/day)	2032.90 (772.75)	2103.54 (823.43)	1907.11 (596.5)	1690.56 (246.86)
Protein (g/day)	72.88 (20.73)	88.31 (37.26)	68.18 (25.3)	71.67 (16.97)
Fat (g/day)	83.83 (32.36)	87.13 (44)	72.769 (30.78)	66.25 (16.29)
CHOm (g/day)	229.54 (85.89)	234.8 (84.51)	226.13 (65.43)	198.36 (41.16)
NSP (g/day)	13.39 (4.93)	14.61 (6.27)	11.68 (3.67)	11.52 (2.89)
Fibre (g/day)	19.05 (6.77)	21.48 (8.31)	16.81 (5.13)	17.44 (4.58)
total SCFA (µmol/g)	44.59 (13.12)	43.27 (11.93)	41.53 (7.47)	41.46 (12.52)
Acetic (µmol/g)	28.01 (6.26)	25.87 (4.87)	25.43 (3.73)	24.77 (5.02)
Propanoic (µmol/g)	6.69 (4.20)	7.14 (3.47)	6.72 (3.20)	6.79 (5.10)
Isobutyric (µmol/g)	0.86 (0.36)	1.23 (0.59)	0.98 (0.31)	1.19 (0.44)
Butyric (µmol/g)	6.95 (4.39)	6.50 (4.22)	6.13 (2.84)	6.00 (2.84)
Isovaleric (µmol/g)	1.03 (0.53)	1.49 (0.81)	1.14 (0.46)	1.52 (0.63)
Valeric (µmol/g)	1.06 (1.17)	1.04 (0.61)	1.13 (0.77)	1.18 (0.75)

Supplemental Table S6: Comparison of demographics, psychological scores and markers of disease severity between cases in the clinical and mechanistic studies

	Clinical study cases	Mechanistic study cases	p value
Number of subjects	22	18	-
Age, median (IQR)	57 (41-64)	56 (39-62)	0.96
Female, *n* (%)	17 (77%)	14 (78%)	0.99
HADS-A, median (IQR)	5 (4-10)	8 (4-10)	0.78
HADS-D, median (IQR)	4 (1-6)	4 (1-7)	0.99
PHQ-12 SS, mean (SD)	5 (3-6)	5 (2-6)	0.85
Fever, *n* (%)	18 (82%)	17 (94%)	0.52
Blood in stool, *n* (%)	4 (18%)	4 (22%)	0.84
Vomiting, *n* (%)	5 (23%)	5 (28%)	0.78
Antibiotics taken, *n* (%)	9 (41%)	5 (28%)	0.70

Supplementary Table 7: Comparison of demographics, psychological scores and markers of disease severity between controls in the clinical and mechanistic studies

	Clinical study controls	Mechanistic study controls	p value
Number of subjects	77	48	-
Age, median (IQR)	62 (48-71)	63 (48-71)	0.69
Female, *n* (%)	33 (43%)	20 (42%)	0.91
HADS-A, median (IQR)	5 (3-7)	5 (3-7)	0.95
HADS-D, median (IQR)	3 (1-5)	3 (1-6)	0.67
PHQ-12 SS, mean (SD)	2 (1-4)	2 (1-4)	0.73
Fever, *n* (%)	42 (55%)	24 (50%)	0.67
Blood in stool, *n* (%)	21 (27%)	9 (19%)	0.42
Vomiting, *n* (%)	16 (21%)	12 (25%)	0.70
Antibiotics taken, *n* (%)	25 (32%)	14 (29%)	0.76

Supplementary Table S8: Significant microbiota differences between cases and controls in samples collected less than 40 days after infection. All samples effected by the antibiotic use were excluded from the analysis. The mean relative abundance of each taxa is shown along with the fold change in cases versus controls.

Phylum	Class	Order	Family	Genus	Case	Control	Fold change
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*		13.83 %	5.25 %	0.38
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Collinsella*	10.70 %	4.31 %	0.40
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Eggerthella*	1.82 %	0.18 %	0.10
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Butyricimonas*	0.01 %	0.13 %	11.85
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Porphyromonas*	0.00 %	0.24 %	62.19
Bacteroidetes	Bacteroidia	Bacteroidales	*Prevotellaceae*	*Paraprevotella*	0.06 %	0.41 %	7.48
Firmicutes	Bacilli	Lactobacillales	*Enterococcaceae*	*Enterococcus*	0.05 %	0.39 %	8.27
Firmicutes	Clostridia	Clostridiales	*Lachnospiraceae*	*Anaerosporobacter*	0.16 %	0.61 %	3.71
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*		11.66 %	18.22 %	1.56
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Faecalibacterium*	6.06 %	8.45 %	1.39
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00 %	0.11 %	34.95
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Phascolarctobacterium*	0.29 %	0.25 %	0.85
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Megamonas*	0.00 %	0.69 %	579.67
Proteobacteria	Betaproteobacteria				0.48 %	1.97 %	4.12
Proteobacteria	Betaproteobacteria	Burkholderiales			0.45 %	1.80 %	4.03
Proteobacteria	Betaproteobacteria	Burkholderiales	*Alcaligenaceae*	*Sutterella*	0.40 %	1.77 %	4.41
Proteobacteria	Betaproteobacteria	Neisseriales	*Neisseriaceae*	*Neisseria*	0.03 %	0.13 %	4.12
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Klebsiella*	0.00 %	0.26 %	146.38

Supplementary Table 8: Significant microbiota differences between cases and controls in samples collected between 40 to 80 days after infection. All samples effected by the antibiotic use are excluded from the analysis. The mean relative abundance of each taxa is shown along with the fold change in cases versus controls

Phylum	Class	Order	Family	Genus	Case	Control	Fold change
Actinobacteria	Actinobacteria	Micrococcales	*Micrococcaceae*		0.03 %	0.27 %	8.54
Actinobacteria	Actinobacteria	Micrococcales	*Micrococcaceae*	*Micrococcus*	0.00 %	0.01 %	26.96
Actinobacteria	Actinobacteria	Micrococcales	*Micrococcaceae*	*Rothia*	0.03 %	0.25 %	8.17
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Olsenella*	0.00 %	0.14 %	Inf
Bacteroidetes	Bacteroidia	Bacteroidales	*Porphyromonadaceae*	*Barnesiella*	0.18 %	0.58 %	3.13
Firmicutes	Clostridia	Clostridiales	*Peptostreptococcaceae*	*Peptostreptococcus*	0.00 %	0.00 %	1.98
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Coprobacillus*	0.00 %	0.01 %	7.59
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Erysipelothrix*	0.00 %	0.01 %	2.02
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00 %	0.02 %	7.43
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*		0.40 %	0.94 %	2.36
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Phascolarctobacterium*	0.19 %	0.52 %	2.80
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Succiniclasticum*	0.00 %	0.08 %	38.37
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Megamonas*	2.58 %	0.07 %	0.03
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Megasphaera*	0.00 %	0.72 %	593.50
Fusobacteria	Fusobacteriia	Fusobacteriales	*Fusobacteriaceae*	*Fusobacterium*	2.24 %	0.02 %	0.01
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*		0.03 %	0.00 %	0.06
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*	*Burkholderia*	0.03 %	0.00 %	0.06
Proteobacteria	Epsilonproteobacteria	Campylobacterales	*Campylobacteraceae*	*Campylobacter*	0.02 %	0.23 %	13.99
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*	*Haemophilus*	0.06 %	0.59 %	10.31

Supplementary Table 9: Significant microbiota differences between cases and controls in samples collected more than 80 days after infection. The mean relative abundance of each taxa is shown along with the fold change in cases versus controls

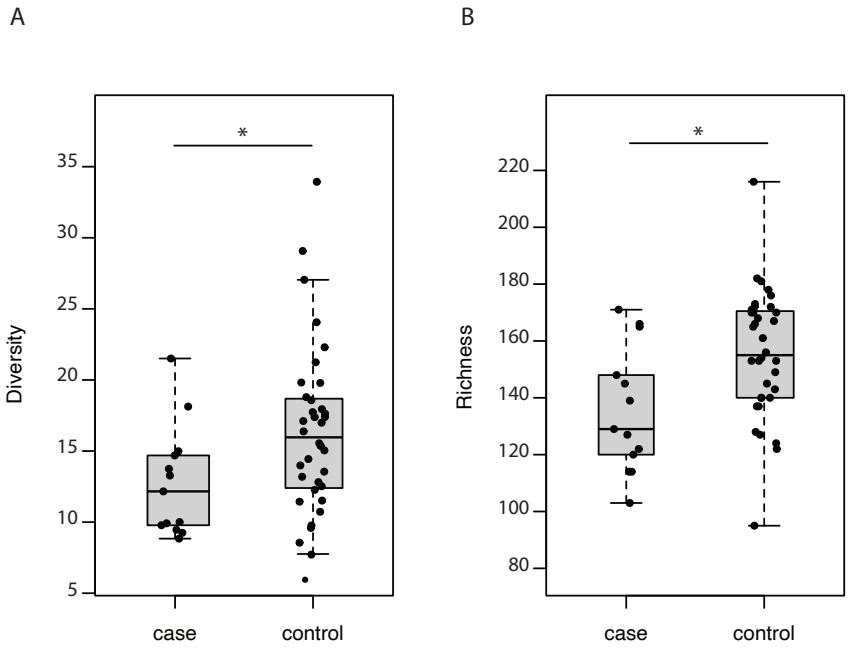
Phylum	Class	Order	Family	Genus	Case	Control	Fold change
Actinobacteria	Actinobacteria	Actinomycetales	*Actinomycetaceae*		0.11 %	0.05 %	0.45
Actinobacteria	Actinobacteria	Actinomycetales	*Actinomycetaceae*	*Actinomyces*	0.11 %	0.05 %	0.45
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*		13.56 %	8.71 %	0.64
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Eggerthella*	2.11 %	0.83 %	0.39
Firmicutes					56.67 %	66.47 %	1.17
Firmicutes	Bacilli	Lactobacillales	*Streptococcaceae*	*Lactococcus*	0.03 %	0.01 %	0.25
Firmicutes	Clostridia	Clostridiales			45.57 %	55.23 %	1.21
Firmicutes	Clostridia	Clostridiales	*FamilyXIIIIncertaeSedis*	*IncertaeSedis*	0.08 %	0.15 %	1.87
Firmicutes	Clostridia	Clostridiales	*FamilyXIIncertaeSedis*	*Peptoniphilus*	0.00 %	0.01 %	12.66
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*	0.10 %	0.25 %	2.49
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*	0.00 %	0.13 %	64.42
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Succiniclasticum*	0.08 %	0.25 %	3.31
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Dialister*	1.97 %	1.25 %	0.64
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Veillonella*	0.10 %	0.25 %	2.53
Fusobacteria	Fusobacteriia	Fusobacteriales	*Fusobacteriaceae*	*Fusobacterium*	0.12 %	0.01 %	0.08
Proteobacteria	Betaproteobacteria	Burkholderiales	*Burkholderiaceae*	*Burkholderia*	0.03 %	0.00 %	0.11
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	*Desulfovibrionaceae*	*Desulfovibrio*	0.02 %	0.14 %	8.67
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*		0.02 %	0.06 %	2.85
Proteobacteria	Gammaproteobacteria	Pasteurellales	*Pasteurellaceae*	*Haemophilus*	0.02 %	0.06 %	2.86

Supplementary Table S11: Significant associations between bacterial taxa and short chain fatty acids. The dietary information was collected from patients visits 2 and 3. Positive association indicated with + and orange, negative association indicated with – and blue.

Phylum	Class	Order	Family	Genus	Total SCFA	Isobutyric	Butyric	Propanoic	Acetic	Valeric	Isovaleric
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Atopobium*					-		
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Collinsella*							+
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Gordonibacter*		+					+
Bacteroidetes	Bacteroidia	Bacteroidales	*Prevotellaceae*						+		
Bacteroidetes	Bacteroidia	Bacteroidales	*Prevotellaceae*	*Paraprevotella*					+	+	
Bacteroidetes	Bacteroidia	Bacteroidales	*Rikenellaceae*	*Alistipes*	-				-		
Firmicutes	Bacilli	Lactobacillales	*Enterococcaceae*		-		-	-			
Firmicutes	Bacilli	Lactobacillales	*Enterococcaceae*	*Enterococcus*	-		-	-			
Firmicutes	Clostridia				-			-	-		
Firmicutes	Clostridia	Clostridiales	*Christensenellaceae*							+	
Firmicutes	Clostridia	Clostridiales	*Christensenellaceae*	*Christensenella*						+	
Firmicutes	Clostridia	Clostridiales	*Clostridiaceae*						-		
Firmicutes	Clostridia	Clostridiales	*Clostridiaceae*	*Clostridium*					-		
Firmicutes	Clostridia	Clostridiales	*FamilyXIIncertaeSedis*		-				-		
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*					-		
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Faecalibacterium*			+				
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Flavonifractor*				-			
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Catenibacterium*		+					+
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*	*Solobacterium*					+		
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Dialister*	+	+		+			
Firmicutes	Negativicutes	Selenomonadales	*Veillonellaceae*	*Mitsuokella*				-			
Proteobacteria	Epsilonproteobacteria								-		
Proteobacteria	Gammaproteobacteria				-		-	-			

Supplementary table S12: Significant associations between bacterial taxa and dietary components. The dietary information was collected from patients visits 2 and 3. Positive association indicated with + and orange, negative association indicated with – and blue.

Phylum	Class	Order	Family	Genus	CHO	Fat	Fibre	NPS	Starch
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Coriobacterium*	+				
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Olsenella*	+				
Actinobacteria	Coriobacteriia	Coriobacteriales	*Coriobacteriaceae*	*Paraeggerthella*	-				+
Firmicutes	Bacilli	Lactobacillales	*Streptococcaceae*					-	-
Firmicutes	Bacilli	Lactobacillales	*Streptococcaceae*	*Streptococcus*				-	-
Firmicutes	Clostridia	Clostridiales	*FamilyXIIIIncertaeSedis*		-			-	
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*Anaerofilum*	-				
Firmicutes	Clostridia	Clostridiales	*Ruminococcaceae*	*IncertaeSedis*		-			
Firmicutes	Erysipelotrichia	Erysipelotrichales	*Erysipelotrichaceae*				-	-	
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*		-				
Firmicutes	Negativicutes	Selenomonadales	*Acidaminococcaceae*	*Succiniclasticum*	-	-			
Proteobacteria	Betaproteobacteria					-			
Proteobacteria	Gammaproteobacteria						-	-	-
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*					-	
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Klebsiella*			-		


A
B
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Diversity
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control

Supplementary methods

The study population

This was a single centre cohort study of patients whose stool sample tested positive for Campylobacter spp. The main clinical study included all 155 eligible subjects who provided clinical details of their illness, psychological parameters, and bowel function. These factors were tested for their association with subsequent post-infective bowel dysfunction (PI-BD). We nested within the main study a detailed mechanistic study of the microbiota in those who provided adequate stool samples. Originally, we had planned to compare just 20 in each group but advances in technology and reduction in costs allowed us to expand our controls and we were able to compare 18 cases with 45 controls, chosen because they had provided the most complete set of stool samples.
The study was approved by the Nottingham Research Ethics Committee on 29th August S (13/EM/0310) and started 1st January 2014. The study was prospectively registered on clinicaltrials.gov (NCT02040922). Although the endpoints were not changed, we initially aimed to recruit 450 to provide adequate power to assess at the impact of antibiotic use during *Campylobacter* infection on prevalence of PI-IBS and to study in depth 20 cases. Despite the fact that we only recruited 129 by the time the funding ceased, and the study closed on Oct 2016, we were able to obtain 18 out of our planned 20 cases for detailed study, whose results are presented here along with the clinical features of 111 controls.

Participants

Inclusion / exclusion criteria
Eligible participants were aged >18, with a clinical syndrome suggestive of intestinal infection, *Campylobacter sp.* in the associated stool sample and living within the Nottingham postal code area. Patients were excluded if they: were pregnant (self-declared), had a pre-existing gastrointestinal disorder or previous resection of the gastrointestinal tract (excluding appendix and gallbladder), had a chronic condition likely to require antibiotics in the next 3 months, regularly used opioids, had used antibiotics or high dose laxatives in the 4 weeks preceding the infection, were unable to complete symptom questionnaires, or were, in the opinion of the investigator, unable to comply with the protocol.

Patient identification
Patients were identified from a stool culture positive for Campylobacter in the Public Health England Laboratory in Nottingham. Data protection laws prevented direct contact so every 2 weeks a batch of letters were sent to patients who had previously been informed of their positive stool culture, inviting them to contact the research team. We hoped to get a sample as early as possible in the illness and to compare this with a sample taken around 6 weeks and 12 weeks from the initial infection, a time span during which our previous study indicated that most people who were going to recover would do so (10).

Patient visits
Visit 1 was arranged as soon as possible after a positive culture, but in the event, there was a considerable delay. Visit 2 was aimed at being 6 weeks after diagnosis though in some cases, owing to delay in making the first appointment this was merged with visit 1 while

Visit 3 was the end of the study 12 weeks after the initial infection. Owing to the delay before patients received the invitation letter the first visit and faecal sample was mean 46 (range 17-93) days and the final sample as mean 97(range 57-160) days from the start of symptoms.

At Visit 1 eligibility was confirmed and written informed consent obtained. Demographics were documented and symptom questionnaires (see below) were completed.

Stool samples

Patients were asked to collect stool samples for each visit, either bringing them within 2 hours of passage to the laboratory for immediate freezing at -80°C or storing at home, double bagged in their domestic freezer at -180C, before bringing in an insulated bag to the laboratory for storage at -80°C prior to analysis.

If Visit 1 occurred within 5 weeks of diagnosis, patients were asked to return for Visit 2 at 6 weeks (typically one week later) to provide a further stool sample. At Visit 3, 12 weeks after diagnosis, patients were asked to complete a questionnaire on their bowel symptoms from the past week and provide a further stool sample

Recruitment

Eligible patients were recruited through the Nottingham University Hospitals Microbiology Laboratory by the Health Protection Team with an invitation letter, information sheet and questionnaires on gastrointestinal symptoms prior to and during the infection. Despite sending out 1286 invitations, recruitment was much slower than expected and after the allotted time of 2 years the study was closed with only 155 patients recruited, of which 99 completed the study (see manuscript Figure 1).

Symptom questionnaires

At Visit 1 patients completed questionnaires documenting demographics prior to infection including prior bowel habit using the Rome III questionnaire, anxiety and depression using the Hospital Anxiety and Depression scale (HADS) [1] and somatisation using the Patient Health Questionnaire -12 Somatic Symptom Scale (PHQ-12 SS) [2]. They were also asked about features of the acute illness with markers of severity including rectal bleeding, weight loss and duration of time off normal activities. We also documented any antibiotic treatment. At each visit they reported their bowel habit during the previous week. At Visit 3, cases were defined as those responding “No” to the question “have your bowels returned to normal” and controls were those who responded “Yes”.

Lack of significant difference between cases with PI-BD and those meeting Rome III criteria

Just under half of the cases (45%) met the Rome criteria III for IBS. These subjects (PI-IBS) were very similar to the remaining 12 not meeting the criteria (PI-BD) with no significant difference in age (mean 57, SD=±14 versus 53 ±15), anxiety (8 ± 6 versus 6 ± 3), depression (median (IQR), 4(2.8-8.5) versus 2(1-7.5)) nor PHQ-12 SS (5(2) versus 4(2)). Neither did the markers of severity differ significantly between PI-BD or PI-IBS, including fever (9/10 versus 9/12), blood in stool (1/10 versus 3/12), vomiting (3/10 versus 2/12) and antibiotic consumption (3/10 versus 6/12).

Microbiota sequencing

The sequencing reads were processed using R package mare and functions ProcessReads and TaxonomicTable. Here the quality filtering, chimera detection, and taxonomic annotation functions rely on USEARCH (version 8.1.1756_i86osx32), [3]. We used only the forward reads for the taxonomical annotation. After removing the primer sequence the reads were trimmed to 180 bases, which were then used for taxonomical assignment. [4] The reads were summed to ASV and reads with less than 68 replicates in all samples were excluded as potentially incorrect and removed from further analysis. The taxonomic annotation was performed using USEARCH by mapping the reads to the SILVA 16S rRNA reference database version 115, curated to contain only sequences matching to the forward and reverse primers that were used for sequencing. No other normalization method was applied after pre-processing. The sequencing controls were assessed and since all the sequencing controls contained less than 1000 reads, the potential contaminants were not removed from the original reads due to very low impact.

Statistical analysis:

In the statistical analysis we used the GroupTest and CovariateTest functions of mare package.[5] The CovariateTest function tests for associations between the desired taxa and a continuous variable. Whereas the group test uses each taxon to test the statistical difference between the studied groupings. Both functions consider the read depth of each sample and uses that as an offset for the model. All taxonomical levels were used as relative data in the analysis from genus level taxa up until phylum level data. The pre-processed data was not transformed in any other way.

There are several statistical models that can be applied in the GroupTest and CovariateTest functions. When having multiple samples from one subject, the subject ID information is used as and will use that variable as the random factor in the model. the function first attempts to fit a zero-inflated negative binomial model using the glmmADMB package. Violation against model assumptions e.g., homogeneity of residuals may lead to meaningless p-values and potentially false conclusions. Therefore, this is considered with in the GroupTest function. If the initial model fails to produce reliable results, glm.nb function, linear models (lm), generalised least squares models (gls), or linear mixed models (lme) are fitted, depending on the situation. If all tests fail, no p-values are given. All obtained p-values are corrected for multiple testing and q-values are produced.

We tested the association between alpha diversity and read counts. The sequenced samples had a median read count of 51893 (lower hinge of 46422 and upper hinge of 57773) indicating that there is not very much difference in the read depth of the sequenced samples. We tested the spearman correlation between read counts and both microbial richness (cor = 0.19, p = 0.03) and diversity (cor = 0.084, p = 0.33). Although the p-value of the correlation between read count and richness is bellow 0.05, the actual association is weak and is due to only few samples (data not shown). We therefore did not see it necessary to adjust the richness and diversity measures with read depth.

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