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.

**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. 3 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 over-growth of the infecting pathogen 4 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 5 and reduces colonisation resistance, typically allowing an overgrowth of both the pathogen and *Proteobacteria*, including facultative anaerobes such as *Enterobacteriaceae*. 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.\textsuperscript{7,8} A resilient microbiota is able to return to its original composition after facing a perturbation, such as an infection, whereas non-resilient microbiota may shift its composition permanently to a new altered state of dysbiosis.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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, \textit{Campylobacter jejuni},\textsuperscript{12} one of the most common causes of bacterial gastroenteritis in Europe.\textsuperscript{13}

Previous pilot studies have shown that PI-IBS following \textit{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.\textsuperscript{14} These findings were replicated in a meta-analysis including an additional PI-IBS group.\textsuperscript{15} Furthermore, similar findings were seen in those who had persistent bowel dysfunction (BD) after \textit{C. jejuni} enteritis but who did not meet Rome criteria (postinfectious BD, PI-BD).\textsuperscript{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 \textit{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.\textsuperscript{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 \textit{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.

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**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). 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 and the Patient Health Questionnaire-12. Somatic Symptom Scale (PHQ-12 SS). 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 V7.00.64) 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 ≥4500 kcal/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.

Microbiota analysis
Facial DNA was extracted using a validated method. 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. The obtained sequence reads (on average 88 213 per sample) were prepossessed with the Mars R package. 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 assign-ment. After preprocessing, there were on average 64 385 reads per sample (range 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 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 (V8.2.1). Normality was tested with D’Agostino’s K² 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 mechan-istic 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 genus level down to bacterial phylum 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 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 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 Bray-Curtis dissimilarities was used to visualise microbial beta-diversity using ASV-level data.
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.

### 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 Questionnaire-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 |
| Number of bowel movements per week (≥3) | 0 (0%) | 3 (4%) | 0 (0 to 2.6) | >0.999 |
| Presence of mucus | 1 (5%) | 1 (1%) | 2.3 (0.4 to 5.1) | 0.397 |
| Sensation of incomplete evacuation | 4 (18%) | 9 (12%) | 1.5 (0.6 to 3.2) | 0.477 |
| 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.

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 significance 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 diversity 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.
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 including Anaerofilum (mean 12.1 in cases vs 15.8 in controls, ANOVA, p=0.015) 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

**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 | Coriobacteria   | Coriobacteriales       | Coriobacteriaceae    |             | 13.61%      | 9.23%          | 1.47        |
| Actinobacteria | Coriobacteria   | Coriobacteriales       | Coriobacteriaceae    |             | 2.59%       | 0.79%          | 3.28        |
| Actinobacteria | Coriobacteria   | 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    | Clostridia      | Clostridiales          | Christensenellaceae  | Christensenella | 0.35%     | 0.71%          | 0.50        |
| Firmicutes    | Clostridia      | Clostridiales          | FamilyXIIIcertaeSedis |             | 0.07%       | 0.19%          | 0.38        |
| Firmicutes    | Clostridia      | Clostridiales          | Ruminococcaceae      |             | 17.76%      | 23.69%         | 0.75        |
| Firmicutes    | Clostridia      | Clostridiales          | Ruminococcaceae      | Anaeroflum   | 0.13%       | 0.28%          | 0.46        |
| Firmicutes    | Erysipelotrichia| Erysipelotrichiales    | Erysipelotrichaceae  | Kandiera     | 0.15%       | 0.10%          | 1.51        |
| Firmicutes    | Erysipelotrichia| Erysipelotrichiales    | Erysipelotrichaceae  |             | 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  | Fusobacteria    | Fusobacteriales        | Fusobacteriaceae     | Fusobacterium | 0.26%       | 0.01%          | 19.32       |
| Fusobacteria  | Fusobacteria    | Fusobacteriales        | Fusobacteriaceae     |             | 0.26%       | 0.01%          | 19.32       |
| Proteobacteria| Betaproteobacteria | Burkholderiales       | Burkholderiaceae     | Burkholderia | 0.04%       | 0.00%          | 16.42       |
| Proteobacteria| Betaproteobacteria | Burkholderiales       | Burkholderiaceae     |             | 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.

**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 Gammaproteobacteria 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 SCFAs production. We found that the decrease in Clostridia was mirrored by the increase in classes such as Gammaproteobacteria and enterotypes 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\(^{15} \) and multiple non-gastrointestinal somatic symptoms 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.
Supplemental material

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