Perioperative changes in the microbiome during rectal cancer surgery: an exploratory analysis of the NIHR IntAct trial

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ABSTRACT

Background:

The gut microbiome may influence post-operative outcomes following rectal cancer surgery, including anastomotic leak. However, perioperative microbiome dynamics and their association with outcomes remain poorly understood. This study aimed to characterize changes in the rectal microbiome in patients undergoing rectal cancer surgery within the NIHR IntAct trial.

Methods:

Rectal swabs were collected at baseline, day of surgery, and post-operative day 3-5. DNA was extracted for 16S rRNA sequencing, and collagenase-producing organisms identified by culture. Associations between microbiome composition and clinical variables were analysed.

Results:

A total of 202 patients were included (mean age 65 years; 69.8% male). At baseline, smoking status explained 3.2% of variation in beta-diversity (p=0.046). On the day of surgery, beta-diversity was associated with hospital site (11.1%, p=0.033), bowel preparation (2.6%, p=0.024), and oral antibiotics (1.0%, p=0.020). Postoperatively, hospital site (16.3%, p<0.001), defunctioning stoma (2.9%, p=0.003) and oral

antibiotics (1.6%, p=0.006) influenced beta-diversity. Alpha-diversity decreased over time, with postoperative increases in *Enterococcus* and *Prevotella*. A defunctioning stoma was associated with lower alpha-diversity, and increased *Pseudomonas* and *Streptococcus*. No significant differences in alpha- or beta-diversity were observed between patients with and without anastomotic leak, although subtle differences in low abundance taxa were detected and 43.9% of post-operative samples demonstrated collagenase activity.

Conclusion:

This is the largest study to date describing perioperative microbiome changes in rectal cancer surgery. Measurable shifts in the microbiome were observed, with small differences between patients with and without anastomotic leak. Further research is needed to explore the clinical significance of these microbiome changes.

LAY SUMMARY

Background:

The gut microbiome is the community of bacteria living in our intestines. These bacteria usually help keep us healthy, but sometimes they cause illness. For people having surgery to remove rectal cancer, a serious complication is when the join between two parts of the bowel does not heal properly, known as anastomotic leak. Changes in the gut microbiome may contribute to this life-threatening problem.

Aim:

This study aimed to understand how the gut microbiome changes before, during, and after rectal cancer surgery, and whether these changes are related to postoperative outcomes, including anastomotic leak.

Methods:

We studied 202 patients having rectal cancer surgery at several NHS hospitals. Samples from the rectum were collected before surgery, during surgery, and a few days after, and analysed to identify the types of bacteria present and their potential effects.

Results:

We found the mix of bacteria changed during treatment. Factors such as smoking, hospital location, bowel cleaning medication, and the use of a defunctioning stoma (a small opening to divert stool) affected the bacterial community. Many patients had bacteria that produce enzymes that may damage tissue. Although there were small differences in the microbiome between patients with and without leaks, no clear link was found.

Conclusion:

Our study shows that the gut microbiome changes during rectal cancer surgery and is influenced by treatment choices. While we found small differences in those with leaks, more research is needed. Understanding these changes in bacteria could help to prevent complications after surgery.

INTRODUCTION

The importance of the gut microbiome is increasingly recognized, with its influence extending across a range of clinical conditions[1]. In rectal cancer surgery, its role in the development of post-operative complications has emerged as an area of growing interest[2]. Among these complications, anastomotic leak remains one of the most feared, due to its association with increased morbidity, mortality, and healthcare costs[3,4]. Despite efforts to reduce known risk factors – such as ensuring a tension-free anastomosis, confirming adequate blood supply, and optimizing surgical technique – the incidence of anastomotic leak remains stubbornly persistent, with reported rates of 10-15% following rectal cancer surgery[5,6].

Pre-clinical studies suggest the gut microbiome can impair anastomotic wound healing[7]. Factors such as surgical injury, ischaemia, and malnutrition appear to disrupt microbial populations, increasing pathogenic bacteria while reducing protective strains[8]. This dysbiosis may promote the colonization of the anastomotic site by pathogenic bacteria, such as *Enterococcus faecalis* and *Pseudomonas aeruginosa*. These organisms produce collagenolytic enzymes and activate host matrix

metalloproteinases, leading to collagen degradation *in vitro* and anastomotic leak in animal models[7]. While these pre-clinical studies provide valuable insights, data from human studies remain limited. Few studies have tracked changes in the microbiome over multiple timepoints in colorectal surgery, and those that have often lack sufficient patient numbers to investigate potential associations with clinical outcomes, such as anastomotic leak[9].

This exploratory analysis, conducted as a sub-study of the NIHR IntAct trial – a European multicenter, randomized, controlled study – aimed to address this gap[10]. Specifically, it sought to: i) describe perioperative changes in the microbiome of rectal cancer surgery patients across defined timepoints, highlighting overall trends within the study population; ii) analyze the microbiome of rectal cancer patients, with an emphasis on key explanatory variables including patient characteristics, disease factors, and treatment approaches; and iii) explore the microbiome profile of rectal cancer patients, with a specific focus on anastomotic leak as a key outcome variable.

METHODS

Study design

A microbiome sub-study was conducted across UK centres participating in the NIHR IntAct trial. IntAct was a multicentre, pan-European, randomized controlled trial that recruited 768 patients across 28 centres in 8 countries. It compared surgery with intra-operative fluorescence angiography (IFA) to standard care (surgery without IFA) to assess the impact on anastomotic leak rates following anterior resection for rectal cancer[10,11]. The microbiome sub-study was limited to UK centres due to logistical constraints – specifically, the need for timely analysis of rectal swabs. Ethical approval was obtained from the Research Ethics Committee (REC) (17/NW/0193) and Health Research Authority (HRA). The trial was prospectively registered with the ISRCTN Registry (ISRCTN13334746).

Sample collection

Rectal swabs (Sigma-Transwabs and M40 charcoal swabs) were collected at three timepoints: 1) baseline (prior to surgery and bowel preparation), 2) day of surgery, and 3) post-operative day 3-5. For patients performing bowel preparation at home, the baseline sample was obtained during a preoperative hospital visit. At each timepoint, a member of the research team collected samples before transferring them for analysis. Samples were analyzed centrally using two approaches: i) 16S rRNA sequencing, and ii) microbial culture to isolate and quantify bacterial collagenase production.

16S rRNA sequencing

DNA was extracted from rectal swabs using the QIAmp DNA Stool Mini Kit[12]. Libraries were prepared following Earth Microbiome Project protocols to amplify the V4 region of the 16S ribosomal gene[13]. Sequencing was performed on Illumina HiSeq3000 and NextSeq2000 (2x150bp reads). Adapters were trimmed with Cutadapt and processed in QIIME2[14,15]. Denoising and merging were done using DADA2[16]. Taxonomy was assigned using QIIME2's BLAST classifier aligned to the SILVA database v132 [17–19].

Alpha-diversity was measured using the Shannon index, which captures richness and evenness of taxa within samples. Beta-diversity was calculated using Bray-Curtis distances, a measure of compositional differences between samples[20]. Associations between beta-diversity and clinical metadata were assessed using permutational multivariate analysis of variance (PERMANOVA) with the adonis2 function in R. To identify associations between specific taxa and clinical variables we used MaAsLin2

(Multivariate Associations with Linear Models) which performs multivariable linear modelling with false discovery rate correction to account for multiple testing[21].

Identification of collagenolytic bacteria and quantification of collagenase production

Collagenolytic bacteria were isolated from rectal swabs using a skim-milk method as previously described[22]. Samples were plated on aerobic and anaerobic media containing skimmed milk and incubated at 37°C. Colonies showing zones of hydrolysis were purified and identified using MALDI-TOF mass spectrometry.

Collagenase activity for all identified collagenolytic isolates was quantified against Type I and Type IV collagen using the EnzChek™ Gelatinase/Collagenase Assay Kit. Isolates were sub-cultured and grown overnight in tryptic soy broth (TSB), then diluted 1:10 in fresh TSB and inoculated into 96 well plates with 25ug/mL collagen substrate and reaction buffer. Florescence (495nm/515nm) was measured over time and quantified against a Clostridium collagenase standard curve (0.06–1U/mL). Absorbance at 595nm was measured to correct for bacterial load.

Clinical data

Clinical metadata were collected for all patients and analysed alongside sequencing and collagenase data. Variables included: sample timepoint, hospital site, age, sex, ethnicity, smoking status, tumour stage and position, neoadjuvant therapy, bowel preparation type, pre-operative oral antibiotics, anastomosis level, defunctioning stoma, circumferential resection margin involvement, indocyanine green (ICG)

administration, and anastomotic leak grade (per the International Study Group definition)[23].

RESULTS

Study population

A total of 202 patients were recruited to the sub-study and provided usable microbiome samples (Table 1). The majority were male (n=141, 69.8%), with a mean age of 65 years (range: 30-89 years). Most did not receive neoadjuvant therapy (n=138, 68.3%). Pre-operative mechanical bowel preparation was administered to all but one patient (n=201, 99.5%), with oral mechanical bowel preparation being the most common (n=151, 74.8%). Seventeen patients (8.4%) also received pre-operative oral antibiotics. A defunctioning stoma was performed in the majority of patients

(n=143, 70.8%). The overall anastomotic leak rate (grades A, B, and C) was 19.8% (n=40).

Sample collection

All 202 patients (100%) were included in the 16S rRNA sequencing analysis, with samples collected at the following timepoints: 98 (49%) at baseline, 180 (89%) intra-operatively, and 103 (51%) post-operatively. In addition, 198 patients (98%) were included in the culture-based microbiome analysis, with samples received at the following timepoints: 101 (50%) at baseline, 198 (98%) intra-operatively, and 110 (54%) post-operatively.

Sequencing metrics

Each sample produced between 1862 and 1,539,340 denoised sequences (median 55,727, mean 83,684). Genera commonly associated with colorectal cancer and healthy stool, including *Bacteroides*, *Prevotella* and *Faecalibacterium,* were prevalent[12,24].

Characteristics of cohort

Initial inspection of the cohort (Figure 1A) revealed that the individual patient was associated with the largest proportion of variation, explaining 66% of beta-diversity ($p<0.001$). Timepoint of sample collection accounted for 3.1% of the variance, and the presence of a defunctioning stoma accounted for 0.5% (both $p<0.001$). Type of mechanical bowel preparation and pre-operative oral antibiotics were associated with non-significant amounts of variation. Together, these five factors accounted for all

clinical variation between samples, making further testing for associations with additional variables redundant.

Perioperative changes in the microbiome across timepoints

Principle coordinate analysis (PCoA) of beta-diversity revealed that baseline and intra-operative samples clustered together, while post-operative samples formed a distinct cluster (Figure 1B). Alpha-diversity decreased between the baseline and intra-operative timepoints, and again between the intra-operative and post-operative timepoints (Figure 1C).

Post-operative samples showed increases in *Enterococcus* and *Prevotella*, and relative decreases in *Faecalibacterium* and *Ruminococcus* (Table S1). Additionally, there was a visible increase in *Pseudomonas* and a decrease in *Bacterioides* between intra-operative and post-operative timepoints. However, these changes were largely influenced by the presence of a defunctioning stoma, and thus, neither of these shifts were significant after multivariate analysis (Figure 1D).

No consistent changes in common taxa were observed between the baseline and intra-operative timepoints (Figure 1D).

Timepoint-specific analysis of the microbiome

At the baseline timepoint (Table 2), smoking status accounted for 3.2% of the variation in beta-diversity ($p=0.046$), but no individual taxa were associated (Table S1).

Notably, no association was observed between beta-diversity and neoadjuvant therapy.

Among intra-operative samples (Table 2), the largest proportion of beta-diversity was explained by hospital site (11.1% of variation, p=0.033), with smaller proportions linked to type of mechanical bowel preparation (2.6% of variation, p=0.024) and pre-operative oral antibiotics (1% of variation, p=0.020). Patients who had rectal enema bowel preparation only had higher levels of *Ruminococcus* compared to those who received oral mechanical bowel preparation alone. The use of oral antibiotics was associated with relative increases in *Ruminococcus*, *Eubacterium*, *Lachnospiricaea* and *Bifidobacterium* (Table S1).

Among post-operative samples (Table 2), beta-diversity was associated with the hospital site (16.3% of variation, p<0.001), the presence of a defunctioning stoma (2.9% of variation, p=0.003) and the use of pre-operative oral antibiotics (1.6% of variation, p=0.006).

Impact of defunctioning stoma on the microbiome

Post-operative samples from patients with a defunctioning stoma showed distinct clustering in PCoA of beta-diversity, separate from both baseline and intra-operative samples (Figure 2B). In contrast, samples from patients without a defunctioning stoma clustered more closely with earlier timepoints. Patients with a defunctioning stoma showed a significant reduction in alpha-diversity (Figure 2C), and notable

increases in *Pseudomonas* and *Streptococcus*, with decreases in *Bacteroides*, *Akkermansia*, and *Parabacteroides* (Figure 2A, table S1).

To investigate whether these differences were attributable to variations in anastomotic height, anastomotic height was included in PERMANOVA analysis. This revealed no significant association between anastomotic height and beta-diversity (P=0.38). Missing data for anastomotic height precluded its inclusion in the original model.

Given the distinct microbiome profile and reduced alpha-diversity observed in post-operative samples from patients with a defunctioning stoma, we further explored changes in key taxa at the individual patient level. We initially focused on the two taxa that showed the most prominent differences, *Pseudomonas* and *Streptococcus*, tracking their changes across timepoints within individual patients (Figure 3A and 3B). In a subset of patients, a marked increase (over 50% of the microbiome) in *Pseudomonas* (13/77, 17%) and *Streptococcus* (4/77, 5%) was observed post-operatively. This spike occurred exclusively in patients with a defunctioning stoma. In the case of *Pseudomonas* spikes, this often accounted for over 70% of the microbiome signal (Figure 3A).

To determine whether other taxa became highly prevalent post-operatively and whether this was associated with the presence of a defunctioning stoma, we plotted the most common taxa in each post-operative sample (Figure 3C). In patients without a defunctioning stoma, 9 out of 26 (34.6%) had a single genus comprising more than

half of the microbiome signal. In patients with a defunctioning stoma, this figure was 31 out of 70 (44.3%). The dominant taxa in these groups differed. *Enterococcus* and *Prevotella* were more common in samples from patients without a defunctioning stoma. These taxa were also seen in samples from patients with a defunctioning stoma, but *Pseudomonas*, *Streptococcus*, *Morganella*, *Aeromonas*, and *Chryseobacterium* were more prevalent.

Anastomotic leak and the microbiome

Focusing on taxa implicated in anastomotic leak in pre-clinical studies, such as *Enterococcus* and *Pseudomonas*, we examined their proportions in patients with and without anastomotic leak. *Pseudomonas* was slightly increased in patients with anastomotic leak, whilst *Enterococcus* was slightly decreased (Figure 4A). Neither change was significant, and both were dwarfed by changes associated with a defunctioning stoma.

Consistent with PERMANOVA analysis, visual inspection of beta-diversity PCoA plots showed no distinct clustering of post-operative samples based on leak status (Figure 4B).

Although anastomotic leak did not significantly affect overall beta-diversity, it was associated with higher relative abundance of *Hungatella* and *Eisenbergiella*, and a reduced abundance of *Barnesiella* on the day of surgery (Table S1); however, all were present at low levels (median <1% per sample). Post-operatively, anastomotic leak

was also linked to an increased relative abundance of *Eubacterium*, although this was less than 0.1% of the signal for most samples (Table S1).

No significant difference in alpha-diversity was observed between patients with and without anastomotic leak (Figure 4C).

Microbiological culture-based analysis of collagenase producing bacteria

To supplement the sequencing data, culture-based methods were used to isolate collagenase producing organisms and quantify their collagenase activity. A total of 401 samples from 198 patients were analysed for collagenase activity. Of these, 152 samples (37.9%) demonstrated evidence of collagenase activity. The proportion of samples with collagenase activity at each timepoint was as follows: 37/101 (36.6%) at baseline, 67/198 (33.8%) intra-operatively, and 48/110 (43.6%) post-operatively. The most commonly identified bacterial species with collagenase activity were: *Pseudomonas aeruginosa* (n=62, 37.7%), *Enterococcus faecalis* (n=33, 19.9%), and *Proteus mirabilis* (n=18, 10.8%) (Table 3).

Following isolation of collagenolytic bacteria, collagenase activity was quantified against Type I and Type IV collagen. The results revealed considerable variability in the collagenolytic potential of these bacteria, even among isolates of the same species. For *Enterococcus faecalis*, type I collagen activity ranged from 30 to 1320 RFU/sx10-3 (mean 277), while for *Pseudomonas aeruginosa* it ranged from 20 to 1916 RFU/sx10-3 (mean 350). Type IV activity ranged from 7.6 to 920 RFU/sx10-3 (mean 177) for

Enterococcus faecalis, while for *Pseudomonas aeruginosa* it ranged from 5.8 to 2086 RFU/sx10-3 (mean 355).

Collagenase activity (as determined by the presence/absence of activity on skim-milk plates), was detected in 11/21 (52%) of post-operative samples with anastomotic leak and 33/77 (43%) of post-operative samples with no leak. This was not significant (Fisher's exact test, p=0.46). Neither type I (Mann-Whitney, p=0.59) or type IV (Mann-Whitney, p=0.58) activity was different between post-operative samples with and without anastomotic leak.

Concordance between 16S rRNA sequencing and microbiological culture data

There was good concordance between culture results and 16S rRNA sequencing data (Table 3). As 16S reads were at the genus-level, taxa were adjusted accordingly. Of the 13 collagenolytic genera identified, eight showed significantly higher 16S read proportions in culture-positive samples. When comparing 16S read abundance across samples with collagenase activity (regardless of organisms), several genera were elevated, but only *Pseudomonas* remained significant after adjusting for multiple testing (Table 3).

DISCUSSION

This study provides valuable insights into the rectal microbiome of patients undergoing rectal cancer surgery, highlighting key factors associated with its composition and the changes that occur during the perioperative period.

Patient-specific variability accounted for the largest proportion of beta-diversity, emphasizing the substantial individual differences in microbiome composition. These differences may be driven by a range of factors, including diet, environmental influences, host genetics, and previous microbial exposures[25]. The timing of sample collection was the second largest contributor to microbiome variation. As patients progressed through treatment, alpha-diversity decreased – typically a marker of a less healthy microbiome [26]. This likely reflects disruption from bowel preparation, surgery, and associated interventions. The reduction in alpha-diversity was accompanied by increased abundance of taxa such as *Enterococcus* and *Prevotella* post-operatively.

Smoking status was associated with variation in the baseline microbiome. Smokers exhibited distinct microbiome profiles, possibly due to smoking-induced changes such as elevated pH, low-grade inflammation, and oxidative stress[27]. In contrast, neoadjuvant therapy explained very little variation in beta-diversity at baseline. Mechanical bowel preparation was another relevant factor, particularly on the day of surgery. Differences were seen between rectal enema and oral preparations, in contrast to previous findings by Zukauskaite *et al*. The use of preoperative oral antibiotics also showed associations with microbiome composition, both on the day of surgery and post-operatively. However, only 8.4% of the study population received oral antibiotics, which may not accurately reflect current clinical practices in light of growing evidence in favor of their use [28]. The small number of patients who received oral antibiotics also limited our ability to further investigate microbiome differences based on the specific type of antibiotic used, an area that warrants further research.

Another observation was the variation in microbiome composition by hospital site on both the day of surgery and post-operatively. The reason remains unclear but could be related to differences in patient populations, local practices, or perioperative antibiotic prescribing protocols. Similar site-specific variation has been reported in other clinical settings[29]. We also observed that the presence of a defunctioning stoma was linked to a distinct post-operative microbiome profile, characterised by reduced alpha-diversity and increased abundance of *Pseudomonas* and *Streptococcus*. The cause for this remains uncertain, though it is possible that the defunctioning stoma could serve as a surrogate for other unmeasured variables influencing the microbiome. We specifically

considered this with respect to anastomotic height within our model, but this did not explain the observed differences, suggesting that this may be driven by factors not yet fully understood. Collectively, these results suggest that despite strong individual variability, the microbiome appears responsive to several modifiable perioperative factors – offering potential avenues for clinical optimisation.

Despite pre-clinical evidence linking the microbiome to anastomotic leak, we found no statistically significant difference in alpha or beta-diversity between patients who developed an anastomotic leak and those who did not. Although some differences in key taxa were observed, such as increases in *Hungatella* and *Eisenbergiella* intra-operatively, and *Eubacterium* post-operatively, as well as decreases in *Barnesiella* intra-operatively, these taxa were all present at relatively low levels, making the clinical significance of these data unclear.

A key aspect of our study was the integration of culture-based analysis allowing us to assess collagenase-producing bacteria. Pre-clinical studies have shown that collagenolytic enzymes play a crucial role in structural degradation of the anastomotic site. Guyton *et al*. demonstrated the utility of skim-milk plates and a collagenase assay to isolate collagenolytic organisms and assess their collagenase production in four patients with anastomotic complications[22]. To our knowledge, this is the first time such methods have been used in a larger clinical study. In our study, we identified *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Proteus mirablis* as the most commonly detected collagenase-producing organisms. These bacteria have been directly implicated in anastomotic leak in pre-clinical studies[7,30,31]. Additionally, we

identified several less common bacterial species from clinical samples that exhibited collagenase activity, suggesting that this virulence factor may extend to other bacteria not previously associated with anastomotic leak.

Notably, we observed substantial variation in the collagenolytic potential of these organisms (as measured by activity against type I and type IV collagen), even among different isolates of the same species. This variability highlights the complexity of understanding the microbiome's role in anastomotic leak and underscores the potential limitations of relying solely on sequencing data, which reflects the presence and relative abundance of bacteria but does not capture important phenotypic characteristics.

Although collagenase activity was detected in a substantial proportion of post-operative samples (43.2%), no clear link with anastomotic leak was found. Nonetheless, the high prevalence of these organisms raises the possibility of preferential colonization at the anastomotic site in patients who develop leak – a question we could not address, as the anastomosis was not directly sampled. Preclinical studies suggest the anastomotic environment can act as a chemoattractant for collagenase producing organisms[32]. Further investigation in human subjects is warranted, though direct sampling poses practical and ethical challenges and will require careful planning. If site-specific colonisation is confirmed, this could inform targeted antimicrobial or microbiome-modulating strategies aimed at reducing anastomotic leak.

Few studies have explored the potential relationship between the microbiome and clinical outcomes in colorectal surgery. Van Praagh *et al*. analysed the mucosal microbiome of anastomotic donuts from stapled colorectal anastomoses in 123 patients as part of the C-seal trial. They found that anastomotic leak was associated with low microbial diversity and high abundance of *Bacteroidaceae* and *Lachnospiraceae*. Shogan *et al*. conducted a prospective study of 101 patients undergoing colorectal resections, collecting samples pre-operatively and on post-operative day 2. They observed that patients who developed post-operative ileus had increased abundance of *Bacteroides*, *Parabacteroides*, and *Ruminococcus*[33]. However, they found no significant microbiome differences in patients who developed surgical site infections or anastomotic leaks.

The strengths of our study include it being the largest study to date on the microbiome of rectal cancer patients undergoing surgery. It was conducted within a multicentre randomised controlled trial with rigorous follow-up data, including the assessment of anastomotic leak via contrast radiology. The study does have limitations. The data should be interpreted within the confines of the study population, which included a high proportion of white ethnicity, and had an unexpectedly high circumferential resection margin positivity rate (19.8% versus <5% in the overall IntAct population). This may be partly due to a higher proportion of low anterior resections in the sub-study. However, inter-site differences in imaging, staging, or operative technique, may also have contributed. Another limitation is the fixed timing of microbiome sampling, which may have missed dynamic microbial changes in patients who developed anastomotic leak after the post-operative sampling window. Future studies

should consider incorporating additional sampling timepoints closer to the time of leak diagnosis to better capture the evolving microbiome in this setting.

In conclusion, patients undergoing rectal cancer surgery in this study demonstrated measurable microbiome changes during the perioperative period. Factors such as smoking, bowel preparation, hospital site and defunctioning stoma had a notable impact on beta-diversity. Alpha-diversity decreased during treatment, with postoperative increases in *Enterococcus* and *Prevotella*. Although small differences in the microbiome were observed between patients with and without anastomotic leak, their clinical significance is unclear and requires further investigation. Importantly, the detection of collagenase producing organisms – previously implicated in leak in pre-clinical studies – merits further exploration, particularly regarding their potential for anastomotic colonization. These findings offer a foundation for future mechanistic and interventional studies aimed at optimizing the microbiome prior to surgery and should be validated in external cohorts.

Figure legends

Figure 1. Microbial profiling of the study cohort.

A. Adonis PERMAOVA analysis of Bray-Curtis beta-diversity across all metadata categories. R^2 values represent the proportion of variation explained; *** indicates $p<0.001$.

B. Principle coordinate analysis (PCoA) plot of Bray-Curtis distances, with samples coloured by timepoint.

C. Shannon alpha-diversity by timepoint. Mann-Whitney test p-values are given.

D. Cumulative relative abundance of bacterial taxa across all samples, grouped by timepoint.

Figure 2. Impact of defunctioning stoma on the post-operative microbiome.

A. Cumulative relative abundance of the most abundant bacterial taxa, stratified by the presence of absence of a defunctioning stoma.

B. Principle coordinate analysis (PCoA) of Bray-Curtis beta-diversity, grouped by timepoint and stoma status.

C. Shannon alpha-diversity of post-operative samples, stratified by stoma status.

Figure 3. Post-operative spikes in specific taxa associated with defunctioning stoma.

A. Relative abundance of *Pseudomonas* across timepoints, stratified by stoma status.

B. Relative abundance of *Streptococcus* across timepoints, stratified by stoma status.

C. Proportion of most abundant genus in each post-operative sample, grouped by presence of absence of a defunctioning stoma.

Figure 4. Comparison of the post-operative microbiome by anastomotic leak status.

A. Cumulative relative abundance of bacterial taxa in post-operative samples, grouped by presence of absence of an anastomotic leak.

B. Principle coordinate analysis (PCoA) of Bray-Curtis beta-diversity, stratified by timepoint and leak status.

C. Shannon alpha-diversity of post-operative samples, grouped by leak status.

Supplementary tables

Table S1: Results of MaAsLin2 multivariate comparison of bacterial taxa to metadata categories. Only taxa with significant associations are shown. For each, the genus, metadata category, the metadata value, coefficient (positive indicates gain of that genus in with that value), standard error, sample numbers, p-value and q-value are shown.

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