Comparison of bacterial quantities in left and right colon biopsies and faeces

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To compare quantities of predominant and pathogenic bacteria in mucosal and faecal samples.

METHODS: Twenty patients undergoing diagnostic colonoscopy with endoscopically and histologically normal mucosa were recruited to the study, 14 subjects of which also supplied faecal (F) samples between 15 d to 105 d post colonoscopy. Mucosal biopsies were taken from each subject from the midportion of the ascending colon (right side samples, RM) and the sigmoid (left side samples, LM). Predominant intestinal and mucosal bacteria including clostridial 16S rRNA gene clusters IV and XIV, Bacteroidetes, Enterobacteriaceae, Bifidobacterium spp., Akkermansia muciniphila (A. muciniphila), Veillonella spp., Collinsella spp., Faecalibacterium prausnitzii (F. prausnitzii) and putative pathogens such as Escherichia coli (E. coli), Clostridium difficile (C. difficile), Helicobacter pylori (H. pylori) and Staphylococcus aureus (S. aureus) were analysed by quantitative polymerase chain reaction (qPCR). Host DNA was quantified from the mucosal samples with human glyceraldehyde 3-phosphate dehydrogenase gene targeting qPCR. Paired t tests and the Pearson correlation were applied for statistical analysis.

RESULTS: The most prominent bacterial groups were clostridial groups IV and XIV and Bacteroidetes and bacterial species F. prausnitzii in both sample types. H. pylori and S. aureus were not detected and C. difficile was detected in only one mucosal sample and three faecal samples. E. coli was detected in less than half of the mucosal samples at both sites, but was present in all faecal samples. All detected bacteria, except Enterobacteriaceae, were present at higher levels in the faeces than in the mucosa, but the different locations in the colon presented comparable quantities (RM, LM and F) followed by P; for RM vs F, P; for LM vs F and P; for RM vs LM: 4.17 ± 0.60 log10/g, 4.16 ± 0.56 log10/g, 5.88 ± 1.92 log10/g, P; = 0.11, P; = 0.0069, P; = 0.9778 for A. muciniphila; 6.25 ± 1.3 log10/g, 6.09 ± 0.81 log10/g, 8.84 ± 1.38 log10/g, P; < 0.0001, P; = 0.0002, P; = 0.6893 for Bacteroidetes; 5.27 ± 1.68 log10/g, 5.36 ± 2.06 log10/g, 8.20 ± 1.14 log10/g, P; < 0.0001, P; < 0.0001, P; = 0.7535 for Bifidobacterium spp.; 6.44 ± 1.15 log10/g, 6.07 ± 1.45 log10/g, 9.74 ± 1.13 log10/g, P; < 0.0001, P; < 0.0001, P; = 0.637 for Clostridium cluster IV; 6.65 ± 1.23 log10/g, 6.57 ± 1.52 log10/g, 9.13 ± 0.96 log10/g, P; < 0.0001.

Abstract

AIM: To compare quantities of predominant and pathogenic bacteria in mucosal and faecal samples.

METHODS: Twenty patients undergoing diagnostic colonoscopy with endoscopically and histologically normal mucosa were recruited to the study, 14 subjects of which also supplied faecal (F) samples between 15 d to 105 d post colonoscopy. Mucosal biopsies were taken from each subject from the midportion of the ascending colon (right side samples, RM) and the sigmoid (left side samples, LM). Predominant intestinal and mucosal bacteria including clostridial 16S rRNA gene clusters IV and XIV, Bacteroidetes, Enterobacteriaceae, Bifidobacterium spp., Akkermansia muciniphila (A. muciniphila), Veillonella spp., Collinsella spp., Faecalibacterium prausnitzii (F. prausnitzii) and putative pathogens such as Escherichia coli (E. coli), Clostridium difficile (C. difficile), Helicobacter pylori (H. pylori) and Staphylococcus aureus (S. aureus) were analysed by quantitative polymerase chain reaction (qPCR). Host DNA was quantified from the mucosal samples with human glyceraldehyde 3-phosphate dehydrogenase gene targeting qPCR. Paired t tests and the Pearson correlation were applied for statistical analysis.

RESULTS: The most prominent bacterial groups were clostridial groups IV and XIV and Bacteroidetes and bacterial species F. prausnitzii in both sample types. H. pylori and S. aureus were not detected and C. difficile was detected in only one mucosal sample and three faecal samples. E. coli was detected in less than half of the mucosal samples at both sites, but was present in all faecal samples. All detected bacteria, except Enterobacteriaceae, were present at higher levels in the faeces than in the mucosa, but the different locations in the colon presented comparable quantities (RM, LM and F) followed by P; for RM vs F, P; for LM vs F and P; for RM vs LM: 4.17 ± 0.60 log10/g, 4.16 ± 0.56 log10/g, 5.88 ± 1.92 log10/g, P; = 0.11, P; = 0.0069, P; = 0.9778 for A. muciniphila; 6.25 ± 1.3 log10/g, 6.09 ± 0.81 log10/g, 8.84 ± 1.38 log10/g, P; < 0.0001, P; = 0.0002, P; = 0.6893 for Bacteroidetes; 5.27 ± 1.68 log10/g, 5.36 ± 2.06 log10/g, 8.20 ± 1.14 log10/g, P; < 0.0001, P; < 0.0001, P; = 0.7535 for Bifidobacterium spp.; 6.44 ± 1.15 log10/g, 6.07 ± 1.45 log10/g, 9.74 ± 1.13 log10/g, P; < 0.0001, P; < 0.0001, P; = 0.637 for Clostridium cluster IV; 6.65 ± 1.23 log10/g, 6.57 ± 1.52 log10/g, 9.13 ± 0.96 log10/g, P; < 0.0001.
CONCLUSION: Non-invasive faecal samples do not reflect bacterial counts on the mucosa at the individual level, except for bifidobacteria often analysed in probiotic intervention studies.

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Key words: Gastrointestinal microbiota; Mucosa; Faeces; Real-time quantitative polymerase chain reaction; Sampling

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INTRODUCTION

Within the gastrointestinal tract, the bacterial community living dispersed in the luminal content differs from those living on the mucosal surface[1] and reflects the health status of the gastrointestinal tract[2]. The mucosal microbiota, intimately located on the host epithelium, has an active role in the host’s immunity and forms an essential part of the protective mucosal barrier against invading pathogens[3][4]. In general, the same main bacterial groups, Firmicutes, Bacteroidetes and Proteobacteria, dominate on the mucosa and in faeces, with the bacterial families of Ruminococcaceae, Actinobacteria, Prevotellaceae, Porphyromonadaceae, Lachnospiraceae and Bacteroidaceae being characteristic for the mucosal microbiota[5][6].

Durban and colleagues assessed the microbial community from four randomly located, pooled mucosal biopsy samples and faecal samples retrieved from 9 volunteers between 2 wk to 8 wk post colonoscopy[6]. They found that on family level taxonomy the mucosal microbiota was higher in richness and diversity and was presented by a comparatively steep rarefaction curve, whereas on species level taxonomy no clear distinction between the two sample types was seen. This could imply that the mucosal environment allows for a variety of microbes to thrive with less exhaustive competition and that, in faeces, the niches are less compartmentalized and thus the most efficiently growing bacterial families dominate. Although both types of microbiota were predominant in Firmicutes and Bacteroidetes, the microbial composition was clearly more dependent on the sample type (biopsy or faeces) than the individual being sampled and the mucosal microbiota was found to be underrepresented in the faecal samples[6].

Hong et al[5] recently published a study in which they applied an elaborate sampling schema which enabled the comparison of closely (1 cm apart) and distantly (left and right colon) located mucosal biopsies from 5 (five) subjects. Unexpectedly, the microbiota on the mucosal surface appeared to be unique, even when comparing closely situated sampling sites (1 cm distance)[5], even though the intestinal microbiota had previously been shown to be subject-specific in several studies[5][7][9]. Thus the study by Hong et al[5] raises further concerns regarding the representativeness of mucosal samples from a certain anatomical location and of faecal samples in relation to the overall mucosal microbiota. Possibly a single mucosal biopsy gives a less reliable picture of the status of the overall gastrointestinal tract than a faecal sample, as faeces represents an end-point view of the ecosystem.

Clearly, for a thorough evaluation of the species composition of the mucosa, faecal material is not a representative sample. However, in many cases the alterations in the quantities of selected bacterial groups or species in the gastrointestinal tract are of interest and, in such a setting, the alterations in bacterial quantities at different mucosal locations and in faeces may be more uniformly expressed, depending on the target species. Thus, the present study focused on the quantification of selected gastrointestinal bacterial groups or species being either dominant, potentially pathogenic, or often encountered on the mucosal surface.

MATERIALS AND METHODS

Subjects

Twenty patients (8 men and 12 women, aged 61 ± 15 years, range: 33-85 years), who underwent colonoscopy between June 2010 and Feb 2011 at the Sahlgrenska University Hospital Östra, Gothenburg, were included in the study. Colonoscopy was performed due to various abdominal complaints, such as diarrhoea, constipation and/or abdominal pain as well as lower gastrointestinal bleeding and/or iron-deficiency anaemia (Table 1). The prerequisite for inclusion into the study was normal-appearing mucosa in the entire colon, and thus patients with any significant pathology, such as colonic polyps, inflammatory bowel disease, malignancy, ischemic colitis etc., were excluded. The possibility of microscopic colitis

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was ruled out through light microscopic examination of biopsy specimens obtained from the mid-portion of the colon ascendens, as well as from the sigmoid. On the other hand, the presence of colonic diverticula was accepted provided there were no signs of acute diverticulitis and/or diverticulosis-associated colitis. Eight tissue specimens for analysis were obtained from the midportion of the ascending colon, as well as from the sigmoid colon, using regular biopsy forceps. One of these specimens from each site was used for analysis of the microbiota. There were no complications related to the colonoscopy or biopsy procedures. In addition, faecal samples were collected post-colonoscopy (15 d to 105 d and unknown for 6 subjects) from 14 subjects. The ethics committee of the University of Gothenburg approved the study and written informed consent was obtained from each of the patients.

### Isolation of DNA and microbial quantification

Bacterial DNA was extracted from the mucosal and faecal samples with the Promega Wizard® Genomic DNA Purification Kit, A1125, (Promega Corporation, Madison, WI, United States) with some minor modifications applied. The mucosal samples were cut in half with scalpel knives and DNA was extracted from both pieces. Homogenisation of the samples was done by bead beating for 3 × 30 s at 6800 g in a 1.4 mL Bertin VK01 glass bead tube, before continuing according to the protocol. Extraction of bacterial DNA from faecal samples was performed as described previously. The DNA concentrations were measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States) and samples were stored at -20 °C until quantitative polymerase chain reaction (qPCR) analysis.

The qPCR reactions were performed using Applied Biosystems Real-Time PCR system equipment (7500 Fast, Applied Biosystems, Foster City, CA, United States) and software applying in-house optimized assay conditions for the primer sequences presented in Table 2. Reactions were run in a 25 μL volume, except for the *Helicobacter pylori* (H. pylori) and *Clostridium difficile* (C. difficile)-targeting qPCR analysis, which were run in a 15 μL volume. Mucosal or faecal microbial DNA was applied as template in quantities of 25 ng or 5 ng respectively. All reactions were run in triplicate. For the human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene assay, 5 ng of mucosal microbial DNA was used as template. In order to obtain standard curves, ten-fold serial dilutions ranging from 1 pg to 10 ng of the genomic DNA of selected bacterial species or human DNA (Table 2) were used. Results were expressed as log_{10} genomes per gram of sample (wet weight), taking into account the size and the 16S rDNA copy number of the standard species genome.

### Statistical analysis

For mucosal samples, the proportion of host DNA was estimated according to the GAPDH qPCR result and subtracted prior to calculations. Outlier values and target bacteria that were not normally distributed due to too low prevalence were removed from the data set. Normality of the data was checked with the D’Agostino and Pearson omnibus K² test and comparisons within bacterial groups between sampling sites were done using paired t tests. Correlations between different sample types for each qPCR assay were analysed using Pearson’s correlation coefficient. Statistical analysis were performed with Prism 5 Version 5.01 (GraphPad Software, Inc., San Diego, United States).

| Patient No. | Age | Gender | Days passed | Reason for referral to colonoscopy | Diverticulosis |
|-------------|-----|--------|-------------|------------------------------------|---------------|
| 1           | 53  | F      | 105         | Iron deficiency anaemia            | Yes           |
| 2           | 41  | M      | NA          | Constipations                      | No            |
| 3           | 43  | M      | NA          | Functional diarrhoea               | No            |
| 4           | 64  | M      | 98          | IBS                                | No            |
| 5           | 85  | M      | NA          | Rectal bleeding                    | Yes           |
| 6           | 75  | M      | 15          | Iron deficiency anaemia            | Yes           |
| 7           | 63  | M      | NA          | IBS                                | No            |
| 8           | 62  | F      | 29          | IBS, constipation                  | No            |
| 9           | 81  | M      | NA          | Iron deficiency anaemia            | Yes           |
| 10          | 72  | F      | 23          | IBS, diarrhoea                     | Yes           |
| 11          | 41  | F      | 21          | Rectal bleeding                    | Yes           |
| 12          | 74  | F      | 26          | Iron deficiency anaemia            | Yes           |
| 13          | 75  | F      | 26          | Follow-up after diverticulosis      | Yes           |
| 14          | 68  | F      | 19          | IBS, diarrhoea                     | No            |
| 15          | 47  | F      | 19          | Follow-up after diverticulosis      | Yes           |
| 16          | 80  | F      | 32          | Iron deficiency anaemia            | Yes           |
| 17          | 54  | M      | NA          | Rectal bleeding                    | No            |
| 18          | 57  | F      | 21          | Rectal bleeding                    | Yes           |
| 19          | 33  | F      | 24          | Diffuse abdominal pain             | No            |
| 20          | 51  | F      | 28          | Rectal bleeding                    | No            |
| qPCR assay                          | Primers                                             | Chemistry\(^1\)                           | Annealing temperature (°C) | Standard species                        | Primer reference                  | Reaction condition reference |
|------------------------------------|-----------------------------------------------------|-------------------------------------------|-----------------------------|----------------------------------------|-----------------------------------|--------------------------------|
| Akkermansia muciniphila           | CAGCACGTGAAGGTGGGGAC                                | FAST SYBR Green Mastermix; 300 nmol/L each primer | 58                          | Akkermansia muciniphila ATCC BAA-835   | Png et al\(^{[20]}\)               | This study                     |
|                                    | CTTGGCGTGGCTTGCTTCAAT                              |                                            |                             |                                        |                                   |                                |
| Bacteroides                       | GCGGACCGGCGGCACCGGG                                 | Power SYBR Green Mastermix; 300 nmol/L each primer | 65                          | Bacteroides fragilis ATCC 25285        | Nakanishi et al\(^{[21]}\)         | This study                     |
|                                    | GRCCTCTCCTCAGAACC                                  |                                            |                             |                                        |                                   |                                |
| Bifidobacterium spp.              | CAGGCCGATGCTTAACG                                  | FAST qTaqMan Mastermix; 300 nmol/L each primer | 60                          | Bifidobacterium adolescentis JCM 1275 | Mäkivuokko et al\(^{[22]}\)        | Mäkivuokko et al\(^{[22]}\)     |
|                                    | CAGGCCGATGCTTAACG                                  |                                            |                             |                                        |                                   |                                |
| Clonostachium cluster IV           | GCAACAGCAGTGGGAT                                   | SYBR Green Core Reagents; 1.5 nmol/L MgCl\(_2\), 250 nmol/L each primer | 62                          | Clonostachium leptum DSM 753           | Matsuki et al\(^{[26]}\)           | This study                     |
|                                    | CTTCCTCCTCCTGCAATA                                  |                                            |                             |                                        |                                   |                                |
| Clonostachium cluster X IV ab      | GAWGAATATATYCTCAGTAT                                | Power SYBR Green Mastermix; 300 nmol/L each primer | 52                          | Clonostachium bolae DSM 15670          | Song et al\(^{[24]}\)             | Lahtinen et al\(^{[25]}\)       |
|                                    | CTAGCCGCTTGGTACCAA                                  |                                            |                             |                                        |                                   |                                |
| Clonostachium difficile            | TTGGCGGATTACTTCGGTAAAGA                            | FAST SYBR Green Mastermix; 300 nmol/L each primer | 60                          | Clonostachium difficile ATCC 9689      | Lahtinen et al\(^{[25]}\)         | Lahtinen et al\(^{[25]}\)       |
|                                    | CCATCTCTGTACTGGCTACAAT                              |                                            |                             |                                        |                                   |                                |
| Collinsella aerofaciens           | CCCAGCCGAGGAGGAGAT                                 | Power SYBR Green Mastermix; 300 nmol/L each primer | 60                          | Collinsella aerofaciens ATCC25986      | Kassinen et al\(^{[26]}\)         | This study                     |
|                                    | CTGTGCACTAGCTACGGTGA                                |                                            |                             |                                        |                                   |                                |
| Domain bacteria                    | CATRHYGTCGTAGCTCGT                                | FAST SYBR Green Mastermix; 200 nmol/L each primer | 60                          | Enterococcus faecium DGCC 2063         | This study                        | This study                     |
|                                    | GGCCGTGTGCTTCAAGCRCC                                 |                                            |                             |                                        |                                   |                                |
| Enterobacteriaceae                 | TGCCGTAACTTCGGGAGAAGGCA                             | SYBR Green Core Reagents; 2 nmol/L MgCl\(_2\), 200 nmol/L each primer | 58                          | Enterococcus faecium DGCC2063          | Matsuda et al\(^{[25]}\)          | This study                     |
|                                    | TCAAGGACCTGTTGCTAAGC                               |                                            |                             |                                        |                                   |                                |
| Escherichia coli                   | ACTGGAATACATTCGGATACAGTAC                           | FAST qTaqMan Mastermix; 100 nmol/L each primer, 30 nmol/L probe | 60                          | Escherichia coli ATCC 11775            | Kaciková et al\(^{[26]}\)         | This study                     |
|                                    | ATCCCTACAGATCTACGCAGAAA                            |                                            |                             |                                        |                                   |                                |
|                                    | CAGCATGCTGGTTCAGACATTTCA                            |                                            |                             |                                        |                                   |                                |
| Faecalibacterium prausnitzii       | CCACTTCACGGCACTAG                                  | SYBR Green Core Reagents; 4 nmol/L MgCl\(_2\), 250 nmol/L each primer | 62                          | Faecalibacterium prausnitzii ATCC 27768 | Rinttilä et al\(^{[26]}\)         | This study                     |
|                                    | GTCGCAAGAGATCAGAC                                  |                                            |                             |                                        |                                   |                                |
| Human GAPDH                        | GTGAAGGAGATGCTGATCGAT                              | Power SYBR Green Mastermix; 300 nmol/L each primer | 60                          | Human DNA                              | Png et al\(^{[25]}\)             | This study                     |
|                                    | CGCCCAATACGACACAAATTCA                              |                                            |                             |                                        |                                   |                                |
| Helicobacterium pylori             | GAAGATAATGAGGATATCAAACGAATGAA                     | FAST SYBR Green Mastermix; 400 nmol/L each primer | 58                          | Helicobacter pylori Modified from Rinttilä et al\(^{[26]}\) | This study                        |                                |
|                                    | CATAGATTCCACACTGACTGATAT                          |                                            |                             |                                        |                                   |                                |
| Staphylococcus aureus              | GCGATGATGATGATGATCGT                               | Power SYBR Green Mastermix; 300 nmol/L each primer | 60                          | Staphylococcus aureus ATCC 29213       | Brakstad et al\(^{[26]}\)         | This study                     |
|                                    | AGCCAAGCTTGGACAGACTAAGC                            |                                            |                             |                                        |                                   |                                |
| Veillonella                        | AYCAACACCTGACCTGAC                                  | Power SYBR Green Mastermix; 200 nmol/L each primer | 60                          | Veillonella parvula DSM 2008            | Rinttilä et al\(^{[26]}\)         | This study                     |
|                                    | CGTCGGATTAAACAGAGCCT                                |                                            |                             |                                        |                                   |                                |

\(^{[1]}\text{Manufactured by (Applied Biosystems, Foster City, CA). GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; qPCR: Quantitative polymerase chain reaction.}\)
The two mucosal sites also correlated significantly between the two mucosal sampling sites and faeces (Figure 1), whereas at the individual different bacteria appeared to follow the same trend in the reus per gram of faeces. present in all faecal samples at log less than half of the mucosal samples at both sites, while appearing mucosa. however, had endoscopically and histologically normal anemia, rectal bleeding and diffuse abdominal pain. All, noscopy due to diverticulitis follow-up, iron deficiency all female, aged 47, 74, 57 and 33 and subject to colo three faecal samples. The sample originating from the left side of the colon and ples, all originating from different subjects: one mucosal sample originating from the right colon as grey bars; biopsies from the right colon as white bars with pattern; biopsies from the left colon as grey bars; faecal samples with dark grey bars). The bacterial quantities between the two mucosal samples did not differ according to paired t-tests, whereas the faecal quantities of all analysed bacteria were significantly higher than those detected for either mucosal site (P < 0.05), except for Enterobacteriaceae. The error bars denote the 95% CI.

RESULTS

Preliminary qPCR analysis from six mucosal and three faecal samples, showed an average percentage of human DNA of 60.74% ± 12.26% and 0.02% ± 0.02% respectively. Thus, the proportion of bacterial DNA was not further analysed for faecal samples as they were assumed to demonstrate 100% bacterial DNA. Among the bacterial groups and species analysed in this study, no alterations were detected between the colonic samples originating from the right and left sides of the colon (Figure 1). The clostridial clusters XIVab and IV, Bacteroidetes and Faecalibacterium prausnitzii (F. prausnitzii) were the most abundant bacteria in all sample types.

H. pylori and Staphylococcus aureus were not detected in any of the samples. C. difficile was detected in four samples, all originating from different subjects: one mucosal sample originating from the left side of the colon and three faecal samples. The C. difficile positive subjects were all female, aged 47, 74, 57 and 33 and subject to colonscopic due to diverticulitis follow-up, iron deficiency anemia, rectal bleeding and diffuse abdominal pain. All, however, had endoscopically and histologically normal appearing mucosa. Escherichia coli (E. coli) was detected in less than half of the mucosal samples at both sites, while present in all faecal samples at log 5.92 ± 1.04 genomes per gram of faeces. H. pylori, Staphylococcus aureus (S. aureus), C. difficile and E. coli were not included in the statistical analysis due to low prevalence.

For the whole subject group, the abundances of different bacteria appeared to follow the same trend in the mucosa and faeces (Figure 1), whereas at the individual level, only Bifidobacterium spp. quantities correlated significantly between the two mucosal sampling sites and faeces (Table 3). The two mucosal sites also correlated significantly for the quantities of Bacteroidetes, Clostridium cluster XIVab and F. prausnitzii (Table 3).

DISCUSSION

The right and left segments of the colon show differences in physiology and motility, creating different environments for bacteria in the murine[11] and human[13,19] mucosa. Our aim was to analyse the quantities of predominant gastrointestinal bacteria and putative pathogenic species in relation to the site of mucosal sampling. We studied 20 patients undergoing diagnostic colonoscopy that displayed, both endoscopically and histologically, normal appearing mucosa. In addition, faecal samples were obtained from 14 subjects between 15 d to 105 d post colonoscopy to assess how well a faecal sample can represent the mucosal microbiota with a 16S rRNA gene-based qPCR. Since in whole community analysis (i.e, 16S rDNA pyrosequencing and metagenomics) the abundance data represents relative proportions of the whole with all groups affecting the result, a targeted analysis, such as qPCR, which quantifies the target independently, could allow for a less biased comparison of quantities. This possibly also results in more uniform representation between different mucosal sampling sites.

The selected bacterial quantities analysed in the present study were comparable between the two mucosal sampling sites for each individual, although previous analysis covering the overall mucosal microbiota with higher taxonomic precision have shown definite heterogeneity between different sampling sites in both humans[19] and rodents[11,13]. However, cleansing of the colon prior to colonoscopy may have distorted the mucosal microbiota at the genus level[13] and possible faecal contamination of the mucosal biopsies may diminish the degree of heterogeneity between mucosal biopsy samples. In addition, the 20 subjects that were analysed, had a considerably heterogeneous background in relation to gastrointestinal health and age, possibly resulting in a wide range of detected microbial quantities reducing the sensitivity of comparative analysis. Of the analysed bacterial groups for both mucosal and faecal quantities, only Bifidobacterium spp. correlated significantly between the different sample types (i.e., a high abundance in faeces predicted a high abundance in mucosal samples at both sites and vice versa, although the faecal quantities were on average higher than the mucosal quantities). As Bifidobacterium spp. have previously been associated with both compromised functional gastrointestinal health[17] and, in some studies, with aging[18], the subjects of the present study may present a substantially wide range of abundance for gastrointestinal bifidobacteria, enabling more evident correlation: 6 of the 20 subjects had irritable bowel syndrome or abdominal pain, and the subjects’ ages varied broadly. The two mucosal sites, the midportion of the ascending colon and the sigmoid, were also comparable in terms of Bacteroidetes, Clostridium cluster XIVab and F. prausnitzii for each subject. The wide time range between colonoscopy and faecal sampling post
The intestinal microbiota has been recognized as an important factor in the maintenance of good health and in the prevention of disease and has thus received a steadily increasing amount of attention in research. It has been widely acknowledged that the mucosal and faecal microbiota are not alike and that even closely situated mucosal samples differ from each other. Thus sampling schemes highly affect the outcome when analyzing intestinal bacteria and an important research focus has been to gain a better insight into the selection of the most appropriate methodologies in each setting and to understand how the techniques compare and complement one another.

**Research frontiers**

The aim of the present study was to test whether quantities of distinct bacterial groups, genera or species, as opposed to a whole community analysis, could be quantified. The aim of the present study was to test whether quantities of distinct bacterial groups, genera or species, as opposed to a whole community analysis, could be quantified. Thus sampling schemes highly affect the outcome when analyzing intestinal bacteria and an important research focus has been to gain a better insight into the selection of the most appropriate methodologies in each setting and to understand how the techniques compare and complement one another.

**Innovations and breakthroughs**

Real-time quantitative polymerase chain reaction (qPCR) allows independent
comparison of each target bacterial group, genera and species between the different samples, whereas whole community approaches are restricted to proportional quantities. In the present study, selected gastrointestinal bacterial groups or species being either dominant, potentially pathogenic, or often encountered on the mucosal surface were quantified from three kinds of samples of up to twenty subjects. Distantly situated mucosal sites were found to have comparable bacterial quantities in an individual, whereas the faecal quantities did not reflect mucosal quantities at the individual level for most bacteria.

Applications

With quantitative analysis of selected bacteria, mucosal biopsies taken from different parts of the colon are comparable, allowing less exhaustive biopsy sampling. Faecal samples, however, poorly reflect mucosal quantities. Applications

Terminology

Quantitative real-time PCR is based on detecting the amount of amplified product during each PCR cycle and comparing the detection threshold cycle to a standard dilution series. Primer and probe design allows a vast array of target selection and taxonomic depth to be applied.

Peer review

This study reports the analysis of several bacterial species, including resident selection and taxonomic depth to be applied. The results are interesting, improving knowledge of the microbiome present in the human colon.

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