World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i32.4404

World J Gastroenterol 2012 August 28; 18(32): 4404-4411
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

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

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Supported by Grants from the Swedish Cancer Society and the Swedish State under the LUA-ALF Agreement
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Received: June 8, 2012 Revised: August 3, 2012
Accepted: August 14, 2012
Published online: August 28, 2012

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 ⅩⅣab, *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 ⅩⅣa+b 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_1 for RM *vs* F, P_2 for LM *vs* F and P_3 for RM *vs* LM: 4.17 ± 0.60 $\log_{10}$/g, 4.16 ± 0.56 $\log_{10}$/g, 5.88 ± 1.92 $\log_{10}$/g, $P_1 = 0.011$, $P_2 = 0.0069$, $P_3 = 0.9778$ for *A. muciniphila*; 6.25 ± 1.3 $\log_{10}$/g, 6.09 ± 0.81 $\log_{10}$/g, 8.84 ± 1.38 $\log_{10}$/g, $P_1 < 0.0001$, $P_2 = 0.0002$, $P_3 = 0.6893$ for *Bacteroidetes*; 5.27 ± 1.68 $\log_{10}$/g, 5.38 ± 2.06 $\log_{10}$/g, 8.20 ± 1.14 $\log_{10}$/g, $P_1 < 0.0001$, $P_2 \leqslant 0.0001$, $P_3 = 0.7535$ for *Bifidobacterium spp*.; 6.44 ± 1.15 $\log_{10}$/g, 6.07 ±1.45 $\log_{10}$/g, 9.74 ±1.13 $\log_{10}$/g, $P_1 < 0.0001$, $P_2 \leqslant 0.0001$, $P_3 = 0.637$ for *Clostridium* cluster Ⅳ; 6.65 ± 1.23 $\log_{10}$/g, 6.57 ± 1.52 $\log_{10}$/g, 9.13 ± 0.96 $\log_{10}$/g, $P_1 <$

0.0001, $P_2 \leqslant 0.0001$, $P_3 = 0.9317$ for *Clostridium* cluster XIVa; 4.57 ± 1.44 $\log_{10}$/g, 4.63 ± 1.34 $\log_{10}$/g, 7.05 ± 2.48 $\log_{10}$/g, $P_1 = 0.012$, $P_2 = 0.0357$, $P_3 = 0.7973$ for *Collinsella* spp.; 7.66 ± 1.50 $\log_{10}$/g, 7.60 ± 1.05 $\log_{10}$/g, 10.02 ± 2.02 $\log_{10}$/g, $P_1 \leqslant 0.0001$, $P_2 = 0.0013$, $P_3 = 0.9919$ for *F. prausnitzsii*; 6.17 ± 1.3 $\log_{10}$/g, 5.85 ± 0.93 $\log_{10}$/g, 7.25 ± 1.01 $\log_{10}$/g, $P_1 = 0.0243$, $P_2 = 0.0319$, $P_3 = 0.6982$ for *Veillonella* spp.; 4.68 ± 1.21 $\log_{10}$/g, 4.71 ± 0.83 $\log_{10}$/g, 5.70 ± 2.00 $\log_{10}$/g, $P_1 = 0.1927$, $P_2 = 0.0605$, $P_3 = 0.6476$ for *Enterobacteriaceae*). The *Bifidobacterium* spp. counts correlated significantly between mucosal sites and mucosal and faecal samples (Pearson correlation coefficients 0.62, $P = 0.040$ and 0.81, $P = 0.005$ between the right mucosal sample and faeces and the left mucosal sample and faeces, respectively).

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.

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

Peer reviewer: Alain L Servin, PhD, Faculty of Pharmacy, French National Institute of Health and Medical Research, Unit 756, Rue J.-B. Clément, F-922296 Châtenay-Malabry, France

Lyra A, Forssten S, Rolny P, Wettergren Y, Lahtinen SJ, Salli K, Cedgård L, Odin E, Gustavsson B, Ouwehand AC. Comparison of bacterial quantities in left and right colon biopsies and faeces. *World J Gastroenterol* 2012; 18(32): 4404-4411 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i32/4404.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i32.4404

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 *Ruminococcaeae*, *Actinobacteria, Prevotellaceae*, *Porhyromonadaceae*, *Lachnospiracheae* 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[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

Table 1 Demographic and clinical characteristics of study subjects

Patient No.	Age	Gender	Days passed[1]	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 diverticulitis	Yes
14	68	F	19	IBS, diarrhoea	No
15	47	F	19	Follow-up after diverticulitis	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

[1]From colonoscopy to faeces sampling. F: Female; M: Male; NA: Not analysed; IBS: Irritable bowel syndrome.

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 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[10]. The DNA concentrations were measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States) and samples were stored at -20 ℃ 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 K^2 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).

Table 2 Real-time polymerase chain reaction primers, probes and assay conditions

qPCR assay	Primers	Chemistry[1]	Annealing temperature (℃)	Standard species	Primer reference	Reaction condition reference
Akkermansia muciniphila	CAGCACGTGAAGGTGGGGAC CCTTGCGGTTGGCTTCAGAT	FAST SYBR Green Mastermix; 300 nmol/L each primer	58	*Akkermancia muciniphila* ATCC BAA-835	Png *et al*[20]	This study
Bacteroidetes	GGCGACCGGCGCACGGG GRCCTTCCTCTCAGAACCC	Power SYBR Green Mastermix; 300 nmol/L each primer	65	*Bacteroides fragilis* ATCC 25285	Nakanishi *et al*[21]	This study
Bifidobacterium spp.	CCTGGTAGTCCACGCCGTAA CAGGCGGGATGCTTAACG ATCCAGCATCCACCG	FAST TaqMan Mastermix; 300 nmol/L each primer, 200 nmol/L probe	60	*Bifidobacterium adolescentis* JCM 1275	Mäkivuokko *et al*[22]	Mäkivuokko *et al*[22]
Clostridium cluster IV	GCACAAGCAGTGGAGT CTTCCTCCGTTTTGTCAA	SYBR Green Core Reagents; 1.5 nmol/L MgCl2, 250 nmol/L each primer	62	*Clostridium leptum* DSM 753	Matsuki *et al*[23]	This study
Clostridium cluster XIVab	GAWGAAGTATYTCGGTATGT CTACGCWCCCTTTACAC	Power SYBR Green Mastermix; 300 nmol/L each primer	52	*Clostridium boltae* DSM 15670	Song *et al*[24]	Lahtinen *et al*[25]
Clostridium difficile	TTGAGCGATTTACTTCGGTAAAGA CCATCCTGTACTGGCTCACCT	FAST SYBR Green Mastermix; 300 nmol/L each primer	60	*Clostridium difficile* ATCC 9689	Lahtinen *et al*[25]	Lahtinen *et al*[25]
Collinsella aerofaciens	CCCGACGGGAGGGGAT CTTCTGCAGGTACAGTCTTGA	Power SYBR Green Mastermix; 300 nmol/L each primer	60	*Collinsella aerofaciens* ATCC25986	Kassinen *et al*[26]	This study
Domain bacteria	CATRGHYGTCGTCAGCTCGT GCGGTGTGTRCAAGRCCC	FAST SYBR Green Mastermix; 200 nmol/L each primer	60	*Enterococcus faecium* DGCC 2063	This study	This study
Enterobacteriaceae	TGCCGTAACTTCGGGAGAAGGCA TCAAGGACCAGTGTTCAGTGTC	SYBR Green Core Reagents; 2 nmol/L MgCl2, 200 nmol/L each primer	58	*Enterococcus faecium* DGCC2063	Matsuda *et al*[27]	This study
Escherichia coli	ACTGGAATACTTCGGATTCAGATACGT ATCCCTACAGATTCATTCCACGAAA fam-CAGCAGCTGGGTTGGCATCAGTTATTCG-tamra	FAST TaqMan Mastermix; 100 nmol/L each primer, 30 nmol/L probe	60	*Escherichia coli* ATCC 11775	Kaclíková *et al*[28]	This study
Faecalibacterium prausnitzii	CCCTTCAGTGCCGCAGT GTCGCAGGATGTCAAGAC	SYBR Green Core Reagents; 4 nmol/L MgCl2, 250 nmol/L each primer	62	*Faecalibacterium prausnitzii* ATCC 27768	Rinttilä *et al*[16]	This study
Human *GAPDH*	GGTAAGGAGATGCTGCATTCG CGCCCAATACGACCAAATCTAA	Power SYBR Green Mastermix; 300 nmol/L each primer	60	Human DNA	Png *et al*[20]	This study
Helicobacterium pylori	GAAGATAATGACGGTATCTAACGAATAA CATAGGATTTCACACCTGACTGACTAT	FAST SYBR Green Mastermix; 400 nmol/L each primer	58	*Helicobacter pylori*	Modified from Rinttilä *et al*[16]	This study
Staplylococcus aureus	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	Power SYBR Green Mastermix; 300 nmol/L each primer	60	*Staphylococcus aureus* ATCC 29213	Brakstad *et al*[29]	This study
Veillonella	AYCAACCTGCCCTTCAGA CGTCCCGATTAACAGAGCTT	Power SYBR Green Mastermix; 200 nmol/L each primer	60	*Veillonella parvula* DSM 2008	Rinttilä *et al*[16]	This study

[1]Manufactured by (Applied Biosystems, Foster City, CA). GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; qPCR: Quantitative polymerase chain reaction.

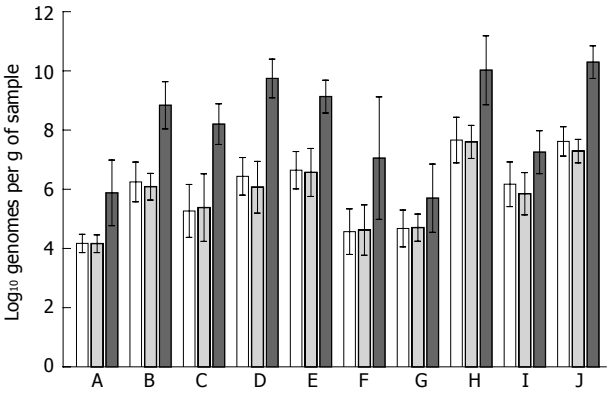

Figure 1 Quantities of bacterial groups detected on the mucosa and in faeces. The bacterial targets A: *Akkermansia muciniphila*; B: *Bacteroidetes*; C: *Bifidobacterium* spp.; D: *Clostridium* cluster IV; E: *Clostridium* cluster XIVab; F: *Collinsella aerofaciens*; G: *Enterobacteriaceae*; H: *Faecalibacterium prausnitzii*; I: *Veillonella* spp.; J: Eubacteria are represented with the three sample types side-by-side (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 colonoscopy 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 $\log_{10}$ 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[1,5] 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[5] and rodents[11,12]. 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[7] and, in some studies, with aging[14], 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

Table 3 Pearson correlations between sample types

Bacterial group/study period	Right colon *vs* left colon		Right colon *vs* faecal sample		Left colon *vs* faecal sample	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Akkermansia muciniphila	0.14	0.63	-0.01	0.97	0.36	0.26
Bacteroidetes	**0.61**	**0.02**	0.45	0.12	0.17	0.61
Bifidobacterium	**0.71**	**0.01**	**0.62**	**0.04**	**0.81**	**0.00**
Clostridium Cluster IV	0.26	0.39	0.26	0.44	0.17	0.64
Clostridium Cluster XIVab	**0.71**	**0.00**	0.54	0.06	0.50	0.09
Collinsella aerofaciens	0.38	0.25	0.63	0.13	-0.87	0.13
Eubacteria	0.19	0.52	0.01	0.97	-0.31	0.33
Enterobacteriaceae	0.38	0.20	**0.59**	**0.03**	0.31	0.35
Faecalibacterium prausnitzii	**0.55**	**0.04**	**0.76**	**0.00**	0.28	0.38
Veillonella	0.33	0.46	0.64	0.09	0.46	0.54

Significant correlations ($P < 0.05$) are designated with bold font.

colonoscopy may bias the correlation analysis. Nevertheless, no statistically significant correlations were found for age, reason for referral to colonoscopy, or for the time that elapsed between colonoscopy and faecal sampling for any of the bacteria analysed (data not shown). Due to the invasive and burdensome nature of colonoscopy, no timely follow-up was possible.

In general, average levels of bacteria were higher in the faeces than in the mucosa and comparable with previously published 16S rRNA gene-targeting qPCR data[15-17]. The clostridial clusters XIVab and IV and *Bacteroidetes* were the most abundant bacterial groups in both sample types, in accordance with the present view of human mucosal and faecal microbiota[5-7,18]. For *Enterobacteriaceae* the higher abundance in faeces was not statistically significant, but a similar trend was evident between the left side mucosal and faecal samples. When analysed in relation to the eubacterial counts (i.e., as proportional values), the majority of the analysed bacteria were as prominent on the mucosa as in the faeces (data not shown), as has previously been shown with RNA-targeting fluorescent in situ hybridization for a selected set of bacterial groups[19]. However, only non-parametric analysis of the target bacteria were possible using proportional values as the data was no longer normally distributed. Nevertheless, even though *Bifidobacterium spp.* was the only bacterial group that correlated within individuals, for the subject group as a whole the average faecal and mucosal bacterial quantities appeared to be associated, as abundant mucosal bacteria were also abundant in faeces (Figure 1). As for the prevalence of the different bacteria, only *Collinsella aerofaciens* was significantly more prevalent on the mucosa (right side of the colon) than in faeces according to Fisher's test (data not shown). *F. prausnitzii* was detected in all sample types with quantities above the eubacterial count ($\log_{10}$ 7.6 ± 1.5, 7.6 ± 1.1 and 10.0 ± 2.0 bacteria per gram of sample for right colon, left colon and faecal sample, respectively; Figure 1), implying technical issues related to the analysis, as it has previously been detected at the level of $\log_{10}$ 8 to 9[16]. The potentially pathogenic bacteria (*H. pylori, S. aureus, C. difficile*) were rarely detected even though 11 of the 20 study subjects were over 60 years of age and all had compromised gastrointestinal health prior to colonoscopy. *E. coli*, which is a common commensal gastrointestinal species, in addition to being a potential pathogen, was more prominent.

Taken together, faecal samples did not reflect quantities of bacteria in the intestinal mucosa at the individual level, except for *Bifidobacterium spp.* which has often been analysed in pro- and prebiotic intervention studies. Although the mucosal microbiota is site-specific in terms of use of community profiling methods, selected bacterial quantities did not differ, even between distant locations in the colon and thus less exhaustive biopsy sampling may be sufficient to evaluate bacterial quantities on the mucosa. At the group level, faecal sampling may be adequate.

ACKNOWLEDGMENTS

Julia Tennilä, Minna Eskola, Jaqueline Flach, Marianne Åkerström, and Ingrid Palmgren are acknowledged for their skilful technical assistance; We also acknowledge Ann-Louise Helminen, Helena Lindegren, Hillevi Björkquist, and Lena Munro for collecting patient samples.

COMMENTS

Background

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 microbiotas are not alike and that even closely situated mucosal samples differ from each other. Thus sampling schemas 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 in a representative manner from mucosal samples originating from different sites in the colon and from faecal samples. Comparable bacterial quantities at different mucosal sites would allow less exhaustive biopsy sampling during colonoscopy while a correlation between mucosal and faecal quantities would allow predictions to be made on the mucosal microbiota from non-invasive faecal samples.

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.

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 and pathogenic bacteria present in the right and left segments of the human colon, compared with species present in faeces. The study is well conducted and the results are interesting, improving knowledge of the microbiome present in the human colon.

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