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Molecular Characterization of Fecal Microbiota in Patients with Viral Diarrhea

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Abstract The study provides molecular analyses of fecal microbiota of diarrhea patients infected with four different types of viruses. Fecal specimens from 52 patients with viral diarrhea (13 each of adenovirus, norovirus, rotavirus, and astrovirus) and six healthy individuals were collected and etiological viral agent was confirmed by enzyme immunoassay and specific PCR. To assess the changes in microbial diversity in patients with viral diarrhea, DNA from stool were extracted and characterized by PCR-denaturing gradient gel electrophoresis (DGGE) with universal primers specific for the V3 region of 16S rRNA gene. The strongest bands of the DGGE profiling were excised and sequenced to identify the dominant groups. *Bacteroides vulgatus*, *Bifidobacterium*, and *Lactobacillus* genera were also enumerated by real time PCR. The results revealed that bacterial diversity and similarity in feces from viral diarrhea groups were significantly lower (mean $H'/\ H'_{max}$ 0.89–0.94, 29–43, respectively) as compared with those of healthy individuals (mean $H'/\ H'_{max}$ 1.36, 59, respectively). Sequencing of dominant bands affirmed that diarrhea groups were mainly comprised of phylum Firmicutes, such as genera *Enterococcus*, *Peptostreptococcaceae incertae sedi*, *Streptococcus*, *Weissella,* and *Clostridium*, and opportunistically pathogenic genus *Shigella*, while dominant group in healthy individuals was phylum Bacteroidetes. Copy number of *Bacteroides vulgatus*, *Bifidobacterium*, and *Lactobacillus* genera was also reduced significantly in viral diarrhea groups as compared to healthy group. It is concluded that opportunistic pathogens increases, while other species of commensal microbiota decrease significantly in the viral diarrhea patients and dysbacteriosis is dependent on type of virus infection.

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Introduction

Diarrhea that can be categorized as acute or chronic is an inflammatory disease of the intestinal tract and is one of the major causes of death in infants and children, especially in developing countries. There are many possible causes of diarrhea in children, but the most common are viruses, such as rotaviruses, noroviruses, adenoviruses, sapoviruses, and astroviruses [3, 14, 26]. The human intestinal microbiota is considered as a "microbial organ" [1, 7, 10] which play a crucial role in nutrition, epithelial development, immunity, defense against pathogens, metabolism, physiology, and gene expression of host [13]. Microbial diversity is essential for the proper role of this so-called microbial organ [10, 35] and changes in its composition have been linked with different metabolic disorders [1, 30] and infections [12]. Diarrhea, induced by viral pathogens can cause dysbacteriosis, changes in the composition of intestinal microbiota and destruction of protective microbial barrier that worsen the illness [4]. It is impelling to analyze the changes in intestinal microbiota in viral diarrhea

patients which can be helpful for the prevention and therapy of viral diarrhea.

Although classical culture-dependent methods can reflect the changes in intestinal microbiota to some extent, these are highly selective, labor-intensive and time-consuming [21]. With the development of culture-independent molecular methods, e.g., fluorescence in situ hybridisation, denaturing gradient gel electrophoresis (DGGE), real time PCR and 16S rRNA gene libraries, a remarkable progress has been made in characterizing the complex microbial communities [5, 18, 24]. DGGE of PCR amplified 16S rRNA genes is now consistently used to evaluate the diversity of microbial communities, and provides a DNA fingerprint of each sample and permits subsequent identification of community members by sequence analysis to monitor their dynamics [5, 30].

The aim of this study was to characterize fecal bacterial diversity in diarrhea patients with four different kind of viral infection and in healthy individuals. PCR–DGGE was combined with image analysis to provide microbial diversity and similarity and sequencing was done to explore the viral diarrhea-associated bacterial taxa. Bacteroides, *Bifidobacterium*, and *Lactobacillus* were enumerated by real time PCR to understand accurate changes in gut microbiota and level of dysbacteriosis caused by different viruses.

Materials and Methods

Samples Collection and Processing

Fifty-two fecal samples were collected from patients infected with four types of viruses (Rotavirus, Astrovirus, Norovirus, and Adenovirus) from 2007 to 2008. The viral antigens from stool samples were detected by specific enzyme immunoassay methods using the RIDASCREEN kit (R-Biopharm, Germany) and confirmed by specific PCR reactions as described previously by Yan et al. [32, 33]. Sample inclusion criteria was (i) the severity of viral infection as determined by the antigen titre of virus (s/co value >6 and <7), (ii) age (2–4 years), (iii) absence of bacterial pathogens in stool and (iv) similar socioeconomic status (same region and having similar dietary habits and income levels of family) of patients. Fecal samples were also collected from six healthy young volunteers as a control group for this study (Table 1). Food habits of healthy control were similar to that of the patients. None of the patients or healthy controls had a history of gastrointestinal diseases before diarrhea, and they did not receive any antibiotics, probiotics, and prebiotics 30 days prior to sampling. All fecal samples were collected using sterile cups and stored immediately at −80°C. The sampling process was performed with the approval of the local Ethics Committee and the informed consent of the patients.

Table 1 Characteristics of the patients in the current study

Virus types	N	Age (years) mean ± S.D.	Sex	
			Male	Female
Control	6	2.73 ± 0.74	3	3
Adenovirus-diarrhea	13	2.77 ± 0.73	6	7
Rotavirus-diarrhea	13	3.21 ± 0.95	8	5
Astrovirus-diarrhea	13	2.98 ± 0.84	7	6
Norovirus-diarrhea	13	3.38 ± 0.99	10	3

Bacterial Strains

Bacteroides vulgatus CICC 22938 and *Bifidobacterium longum* CICC 6186 were obtained from Chinese Center of Industrial Culture Collection (CICC, China), while *Lactobacillus rhamnosus* NWS19 was from our laboratory. These strains were cultured anaerobically in GAM broth (Nissui Seiyaku Co., Ltd., Tokyo) supplemented with 1.0% glucose at 37°C for 24 h and used as standard strains in enumeration of bacteria from stool samples by real time PCR.

Extraction of Total DNA

After the fecal samples were thawed on ice, DNA was extracted using the QIAGEN QIAamp MiniStool kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, with an initial bead-beating step of 30 S at 5,000 rpm. Extracts were treated with DNase-free RNase (100 mg l^{-1}) and DNA concentration was determined by a NanoPhotometer™ (IMPLEN, Germany) [23].

PCR Amplification for DGGE

Total fecal DNAs were used as templates for PCR–DGGE as described previously [30], by using Primers targeting the variable V3 region of 16S rDNA. The nucleotide sequences of the primers are as follows: primer-F: 5′-GC clamp-CCT ACGGGAGGCAGCAG-3′, primer-R: 5′-ATTACCGCGG CTGCTGG-3′. A GC clamp (CGCCCGCCGCGCGCGG CGGGCGGGGCGGGGGCACGGGGGG) was attached to the 5′ end of the forward primer to allow detection of the corresponding PCR products with DGGE [19]. Each 50 μl PCR reaction mixture contained 20 pmol of each primer, 10 mM of each deoxynucleoside triphosphate (dNTP), 2.5 mM $MgCl_2$, 2 U of Taq DNA polymerase (Promega, USA), 10× buffer, and 2 μl of total fecal DNA (120 ng approx.). PCR amplification was done in an automated thermocycler (ABI2720, USA) using touchdown PCR programs: Initial denaturation at 95°C for 5 min, followed

by denaturation at 95°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 1 min. The annealing temperature was decreased by 1°C every second cycle until a touchdown of 55°C, at this temperature ten additional cycles were carried out, followed by final extension at 72°C for 7 min and holding at 4°C. PCR products were resolved in 2% [w/v] agarose gel electrophoresis (300–400 ng DNA per lane). Bands were visualized using ethidium bromide staining (5 μg/ml).

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed using a DCode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA) with gels of size 16 × 10 × 1 mm. Separation of the PCR products was achieved in 10% (w/v) polyacrylamide (acrylamide–bis, 37.5:1) gels in 1× Tris–acetate EDTA (TAE) buffer, containing 40–75% linear denaturant gradient. The 100% denaturing solution contained 7.0 M urea and 40% (vol/vol) deionized formamide. Electrophoresis was done at a constant voltage 100 V and 60°C for 12 h. The gels were stained with a 5 μg/ml ethidium bromide solution for 30 min, washed with deionized water and viewed using Bio-Rad Gel Doc 2000. DGGE profiles in different gels were compared by employing a standard reference (DNA marker: DL2000) and digitally normalized with a standard pattern using the BioNumerics software, version 2.50 (Applied Maths, St.-Martens-Latem, Belgium). All gels were run simultaneously under the same electrophoretic conditions to minimize experimental errors [25].

Recovery of Bands and Sequencing

Dominant bands were excised from DGGE gels using a sterile scalpel and resuspended in 100 μl of Tris–HCl (10 mM l^{-1}, pH 8.0) overnight at 4°C, heated at 99°C for 30 min, and a 4 μl aliquot was subsequently re-amplified by PCR using the original primers without GC clamp. After purification, PCR products were cloned into the p-GEM T Easy vector (Promega, USA) according to the manufacturer's instructions. Competent *Escherichia coli* DH5a cells were transformed and screened for plasmid insertions according to the manufacturer's instructions. Final PCR products were purified using Qiaquick PCR purification kit (Qiagen, Valencia, CA, USA) and were sequenced by Shanghai Sangon Biological Engineering Technology Service Co. Ltd. (Shanghai, China). Sequences were analyzed and their similarity was tested against 16S rRNA sequences stored in GenBank by using BLAST to identify the species or genus.

Statistical Analysis of DGGE Fingerprinting

The bacterial diversity of diarrhea group and healthy control group was evaluated by the number of bands and the band intensity of DGGE profiles using Quantity One software (Bio-Rad, USA). The Shannon and Weaver index of diversity (H′) was used to determine the diversity of taxa present in fecal microbiota from diarrhea group and healthy group [8, 15]. The similarity score of DGGE profiles were performed using the UPGMA method based on the Dice similarity coefficient (band-based).The cumulative distribution curve of four viral diarrhea groups was also drawn on the basis of Dice similarity coefficient [11]. The unpaired *t* test and *F* test were applied to compare the number of bands, diversity H′ index and similarity coefficient among diarrhea and healthy group (P value of <0.05 was interpreted as statistically significant). All statistical analyses were performed using a software SPSS version 13 (Chicago, Ill.).

Direct Enumeration of Bacteria by Real Time PCR

Bifidobacterium, *Lactobacillus*, and *Bacteroides vulgatus* were enumerated by real time PCR by using specific primers (Table 2) in Bio-Rad CFX96 real time PCR detection system (Bio-Rad Laboratories, USA). Each 20 μl reaction mixture contained 10 μl of 2× SYBR Green PCR Master Mix (TOYOBO, Osaka, Japan), 1 μl of each primer (5 μM), 2 μl of sample DNA and 6 μl sterilized ultra pure H_2O. The amplification program consisted of one cycle of 95°C for 5 min, 40 cycles of 95°C for 10 s, 55°C for 15 s and 72°C for 50 s, and finally one cycle of 95°C for 15 s. The fluorescent product was detected at the end of each cycle. After amplification, melting temperature analysis of

Table 2 Primer sets used for real time PCR reactions

Target organism	Primer	Primer sequence (5′ → 3′)	Size (bp)	Reference
Bacteroides vulgatus	BAC.F	GCATCATGAGTCCGCATGTTC	287	[27]
	BAC.R	TCCATACCCGACTTTATTCCTT		
Bifidobacterium	Bifid-F	CTCCTGGAAACGGGTGG	550	[17]
	Bifid-R	GGTGTTCTTCCCGATATCTACA		
Lactobacillus genus	XB5-F	GCCTTGTACACACCGCCCGT	250	[20]
	LbLMA1-R	CTCAAAACTAAACAAAGTTTC		[6]

PCR products was performed to evaluate the specificity of PCR. The melting curves were obtained by slow heating at 0.1°C/s increments from 65 to 95°C, with continuous fluorescence collection. For enumeration of *Bifidobacterium*, *Lactobacillus*, and *Bacteroides vulgatus* in each sample fluorescent signals detected from six serial dilutions were averaged and compared to a standard curve generated from the standard DNA. The standards used for quantifying the sample DNA were generated by amplifying the DNA of standard strains *Bacteroides vulgatus* CICC 22938, *Bifidobacterium longum* CICC 6186, and *Lactobacillus rhamnosus* NWS19 by using specific primers. Amplicons were resolved on agarose gel, excised, purified, and cloned in *E.coli* DH5 alpha by p-GEM T Easy vector according to the manufacturer's instructions. Competent *E. coli* DH5α cells were transformed and screened for plasmid insertions according to the manufacturer's instructions. Plasmids with corresponding inserts were extracted and concentration was determined by NanoPhotometer™ and the copy number was calculated in terms of the product size. Real time PCR experiments were performed in triplicate and mean was used in results.

Results

Analysis of DGGE Profiles of Four Viral-Diarrhea and Healthy Groups

PCR–DGGE analysis with universal primers targeting the V3 region of the 16S rRNA gene was used to analyze the dominant fecal microbiota of four viral diarrhea and healthy group (Fig. 1). The number, position, and intensity of the bands were different among four diarrhea groups as well as in healthy controls, which showed the complex fingerprint of intestinal microbiota. Differences of DGGE fingerprint profiles were also observed among four viral diarrhea groups, which indicates that diarrhea caused by

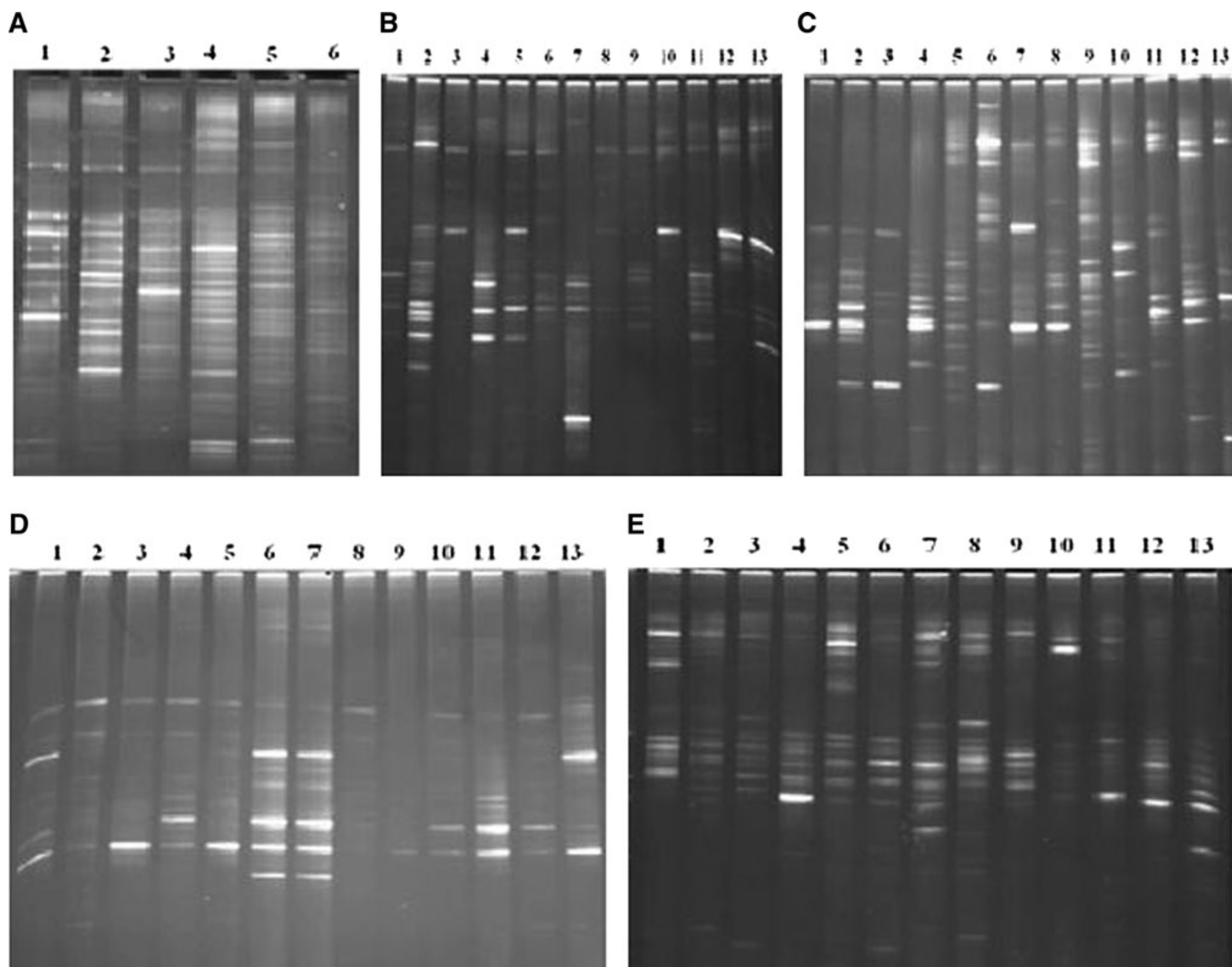

Fig. 1 DGGE profiles of healthy control group and four types of viral diarrhea groups obtained with universal primer (V3): **a** Control group, **b** Adenovirus-diarrhea group, **c** Rotavirus-diarrhea group, **d** Astrovirus-diarrhea group, **e** Norovirus-diarrhea group

different viruses may result in different changes of gut microbiota. Common bands were also observed in different samples of every group.

We calculated the band number of DGGE profiles and Shannon–Weaver diversity index (H') to reflect the diversity of intestinal microbiota in diarrhea groups and healthy individuals (Table 3). The band number and H′ index of viral diarrhea groups were significantly lower as compared with healthy group ($P < 0.05$), which reflects the reduction in the diversity of intestinal microbiota. Although, the difference in band numbers was highest for astrovirus group, significant differences in H' index among viral diarrhea groups were not observed.

Intestinal Microbiota Similarities Analysis of the Different Viral-Diarrhea Groups

Similarity of intestinal microbiota in viral diarrhea and healthy group is presented as a cumulative distribution curve of the pairing similarity coefficient of each group in Fig. 2. The curve showed the distributional characteristics of similarity coefficient. These results indicated that gut microbiota change in viral diarrhea groups are different. The mean values of the band-based Dice similarity coefficient are shown in table (3). Remarkable differences of gut microbiota were observed among virus-diarrhea groups and healthy control groups. Intra group similarity of all viral diarrhea groups was significantly lower as compared with healthy groups ($P < 0.05$). Similarity in microbiota of individuals in astrovirus group (43.37 ± 16.919%) was highest, followed by norovirus (40.6 ± 14.975%), adenovirus (30.99 ± 12.95%), and rotavirus (28.96 ± 13.35%).

Table 3 Microbiota diversity and similarity of diarrhea patients and healthy controls

Group	Microbiota diversity		Microbiota similarity[§]
	Bands number* (Mean ± SD)	Shannon index ratio[‡] (Mean H'/H'_{max} ± SD)	
Control	22.17 ± 5.00[a]	1.356 ± 0.0127[a]	58.6 ± 8.4677[a]
Adenovirus	11.38 ± 4.15[b]	0.898 ± 0.0654[b]	30.99 ± 12.949[b]
Rotavirus	10.69 ± 5.33[b]	0.949 ± 0.05[b]	28.96 ± 13.345[c]
Astrovirus	7.23 ± 2.80[c]	0.971 ± 0.016[a]	43.37 ± 16.919[d]
Norovirus	11.31 ± 4.21[b]	0.929 ± 0.0569[b]	40.6 ± 14.975[e]

Values within the same column followed by different superscript letters differ significantly ($P < 0.05$)

* Numbers of denaturing gel electrophoresis (DGGE) bands produced by each sample were compared by independent t test. A P value < 0.05 was considered as statistically significant

‡ Shannon diversity index (H') was calculated using the relative intensities of all DGGE bands in each sample and expressed as a ratio of H' to H'_{max}, where H'_{max} is the maximum value of the Shannon index for a given sample. Virus-diarrhea groups and healthy controls were compared by independent t test, P values < 0.05 was considered as statistically significant

§ DGGE banding profiles between members of a given group were compared by Dice similarity coefficients. Dice similarity coefficients of virus-diarrhea and healthy control groups were compared by independent t test

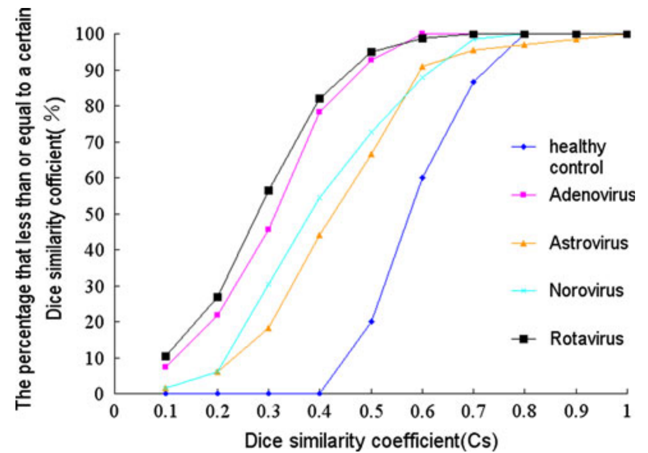

Fig. 2 Accumulative curves of Dice similarity coefficient of the five groups. The x-axis is Dice similarity coefficient (C_s), from 0.0 to 1.0. The y-axis is the percentage that is less than or equal to a certain Dice similarity coefficient, from 0 to 100%

Sequencing Results Analysis of Dominant Band

Dominant bands from DGGE profiles were excised and sequenced. The bands from different positions in DGGE profile were selected for sequencing. A band on the same position in different lanes of a gel was selected only once. Sequence results were used to identify the dominant groups by BLAST. The results showed that phylum Bacteroidetes, Firmicutes, and Proteobacteria were dominant in healthy group, and viral diarrhea groups were comprised of phylum Firmicutes and Proteobacteria mainly. Sequence of dominant bands also revealed that genus *Enterococcus*, *Peptostreptococcaceae Incertae Sedi*, *Streptococcus*, *Shigella*, and *Weissella* were more dominant in viral diarrhea groups. We also sequenced few bands from same position of different lanes of a gel to certify the resolution capability of DGGE and resulting sequences were same (data not shown).

Quantitative PCR Detection of *Bacteroides vulgatus*, Bifodobacteria, and *Lactobacilli*

Real time PCR analyses were performed to quantify *Bacteroides vulgatus*, *Bifidobacterium*, and *Lactobacillus* genus in fecal samples of viral diarrhea and healthy group. The standard plasmid containing corresponding insert for *Bacteroides vulgatus* CICC 22938, *Bifidobacterium longum* CICC 6186, and *Lactobacillus rhamnosus* NWS19,

with six serial dilutions was simultaneously used for each detection. The results indicated that the viral diarrhea groups had significantly lower copy numbers of *Bacteroides vulgatus*, *Bifidobacterium*, and *Lactobacillus* in the fecal microbiota as compared to healthy group ($P < 0.05$) except for copy number of *Bifidobacterium* in rotavirus group that was slightly higher than healthy group (Table 4).

Discussion

The microbial ecology of the gastrointestinal tract includes the diversity of taxa present in the GI tract, their activities, and relationship with each other and the host (synergistic and competitive interactions) [34]. Resident bacteria are a crucial line of defence against the colonization and tissue invasion by exogenous microbes or pathogens [9]. As a result of viral infections, resident microbiota of human gastrointestinal tract goes through prominent changes [16], which can affect the human immunity and physiology beyond the scope of viral infection itself.

Our study showed that the fecal microbial composition was different between the viral diarrhea groups and healthy group by molecular profiling, sequencing of dominant bands from DGGE profile and real time PCR analysis. More specifically, there were significant difference in the similarity of bacterial communities and the number of and *Bacteroides vulgatus* measured by the Dice similarity coefficient and bacterial copy number in viral diarrhea groups as compared to healthy group. Results of this study promote basic knowledge about the intestinal bacterial communities in viral diarrhea. Copy number of *Lactobacilli* and *Bifodobacteria* in feces was also reduced in viral diarrhea groups except for rotavirus-diarrhea group, where *Bifidobacterium* copy number was not affected.

The complex methods required to isolate and identify the intestinal microbiota members and especially the impossibility of culturing many of them have thus far hindered the study of these populations. Advances in microbial ecology and culture-independent methods have proved useful to understand the polymicrobial processes and have highlighted the limitations of culture dependant methods. In this study, the bacterial diversity of the gut microbiota in viral diarrhea groups and healthy group was analyzed by combining DGGE of the 16S rRNA gene with imaging and sequencing of key PCR amplicons, together with statistical analyses. DGGE fingerprint techniques based on the sequence diversity of the 16S rRNA gene have been used to study complex bacterial communities and appear to be ideal for monitoring community shifts and changes [34]. DGGE offers several potential advantages over culture techniques for analysis of anaerobic stool microbiota [19]. Multiple samples can be analyzed simultaneously on a single gel, allowing direct comparisons between samples and bands of interest can be excised and sequenced directly to identify bacterial species [19]. DGGE fingerprint are routinely used now to analyze the molecular characteristic of complex microbial communities [30].

To assess the diversity of intestinal microbiota, we calculated the number of bands and Shannon–Weaver index (H') from DGGE profile and found significantly lower diversity in the diarrhea groups as compared with healthy control group. This result might indicate that virus infection caused intestinal damage and resulted in changes in the structure and composition of intestinal microbiota, further, the damage of gut microbiota might lead to the worsening of the sickness. To analyze the differences of gut microbiota of four types of diarrhea, the similarity of gut microbiota were compared by Dice similarity coefficient and cumulative distribution curves of the pairing similarity coefficient. The result showed that the similarity between individuals decreased to different extent for all diarrhea groups as compared to healthy groups. It indicates that microbiota composition of each individual vary to different degrees as a result of viral diarrhea. Thus, virus infection resulting in diarrhea may seriously affects the composition of intestinal bacteria and the level of dysbacteriosis can be different for different viral agent.

In order to identify dominant bacteria, we selected dominant bands from different positions to sequence from diarrhea groups and healthy control group. The result of sequencing indicated that the dominant microbiota of four types of virus-diarrhea patients was different compared with that of healthy group. Opportunistic pathogens, enterococci and streptococci increased in gut microbiota of viral diarrhea

Table 4 Real time PCR quantification of bacterial copy number

Group	Bacterial copies (mean ± SD)/g of fecal material		
	Lactobacillus	*Bifidobacterium*	*Bacteroides vulgatus*
Control	$(4.7 \pm 3.15) \times 10^5$ [a]	$(9.21 \pm 3.45) \times 10^5$ [a]	$(5.21 \pm 3.45) \times 10^8$ [a]
Adenovirus	$(6.21 \pm 4.65) \times 10^2$ [b]	$(1.09 \pm 2.45) \times 10^4$ [b]	$(1.640 \pm 4.40) \times 10^2$ [b]
Rotavirus	$(3.21 \pm 1.45) \times 10^2$ [b]	$(1.14 \pm 4.09) \times 10^6$ [a]	$(1.20 \pm 2.33) \times 10^2$ [b]
Astrovirus	$(8.45 \pm 5.13) \times 10^2$ [b]	$(4.88 \pm 7.31) \times 10^2$ [c]	$(3.90 \pm 5.45) \times 10^2$ [b]
Norovirus	$(4.56 \pm 2.25) \times 10^3$ [c]	$(3.69 \pm 1.31) \times 10^3$ [d]	$(2.20 \pm 1.45) \times 10^2$ [b]

Values within the same column followed by different superscript letters differ significantly ($P < 0.05$)

patients, while the phylum Bacteroidetes was not dominant in viral diarrhea. Sequencing result of predominant microbiota further proved that virus diarrhea had destroyed the composition and structure of gut microbiota and the balance between beneficial bacteria and opportunistic bacteria. To certify the resolution capability of DGGE, few bands from the same position in different lanes of a gel were also sequenced, which showed that the bands from same position of different lanes of a gel were identical, which indicate that same bacterium have same bands in DGGE map.

The largest proportion of microbes which resides in human intestine falls into two groups, the Bacteroidetes and the Firmicutes [31]. *Bacteroides vulgatus* is the numerically predominant Bacteroides species in the human colonic microbiota which forms a complex but beneficial relationship with the host including the prevention of intestinal colonization [28, 29]. *Lactobacilli* and *Bifidobacteria* are also very important groups of intestinal microbiota as they have many beneficial effects on host [2, 22]. We analyzed the affect of viral diarrhea on *Bacteroides vulgatus*, *Bifidobacterium* and *Lactobacillus* by real time PCR and found that generally the copy number of these bacteria was significantly reduced in diarrhea groups as compared with healthy group. However, *Bifodobacteria* were slightly increased in rotavirus-diarrhea group. In this study, only dominant populations are detected by using PCR-based approach, so it is not a measure of the total diversity of the samples, but instead a measure of relative diversity for comparison between samples. In fact, at this point of time, our focus was to indicate that if there are any differences in intestinal microbiota in viral diarrhea patients. Since we got positive findings, it has opened new avenues to take it to further studies with large number of samples and to see viral associated damage to gut ecological homeostasis as a result of dysbacteriosis.

Conclusion

Taken together, in this study, we observed the characterization of intestinal microbiota in viral diarrhea, which suggests that the intestinal microbiota of viral diarrhea patients have some changes associated with the type of viral infection. Predominant microbiota of four types of viral diarrhea patients shows significant differences from that of healthy individuals, and different viruses may have different influence on the intestinal microbiota.

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