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Gut Microbiome Study in Pediatric Patients with Short Bowel Syndrome Receiving Alternate Gastrointestinal Prophylactic Antibiotics

Le Yi Phyo, Wison Laochareonsuk, Napasorn Phutong, Kaimook Boonsanit, Piyawan Chiengkriwate, Komwit Surachat, Kamonnut Singkhamanan and Surasak Sangkhathat

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Different microorganisms, especially bacteria, viruses, archaea and unidentified various eukaryotes occupy the gastrointestinal tract in human, that has been known as gut microbiota [1]. It is universally accepted that commensal gut microbiota is contributed to the improvement of barrier function in the intestinal epithelium, development of the healthy immunity, the absorption of nutrients and prevention of the pathogens colonization [2]. For this reason, the gut microbiota is recognized as a crucial organ to protect the intestinal homeostasis. Nevertheless, this homeostasis mechanisms can be changed by various factors, particularly the change of diet, stress, infection, intestinal ischemia-reperfusion and antibiotics [3-6]. This results in an imbalance of microbiota known as gut dysbiosis.

Small intestinal bacterial overgrowth (SIBO) is characterized by the microbiota imbalance in the gut due to the impairment of the regular system. The most essential defensive mechanism involves the secretion of gastric acid, gut motility, the function of the ileocecal valve, the secretion of biliary and pancreatic enzyme, and the mucosal immune system. SIBO is an unusually excessive bacterial count in the small intestine accepted more than 10^5 colony-forming unit (CFU)/mL of bacteria, including the prominent of gram-negative aerobes and anaerobic bacteria, by the aspiration of jejunal fluid [7].

The high risk of SIBO development is identified by the main disease of pediatric with intestinal failure (IF) due to the numerous factors, in particular, the gut motility disorders and anatomical abnormalities, ileocecal valve resection and taking antacids [7, 8]. Intestinal failure (IF) is represented as the functional abnormalities of the gastrointestinal tract (the functional gastrointestinal tract abnormalities) provided the homeostasis of electrolyte, adequate hydration, and growth. Short bowel syndrome (SBS) represents the vast majority of pediatric IF. The effect of this might be from the resection of the extensive small bowel by various reasons such as intestinal volvulus or necrotizing enterocolitis (NEC) and congenital disorders, particularly jejunal atresia and gastroschisis. Neonatal SBS resulted in a high incidence of morbidity and mortality [9]. Moreover, patients with severe SBS are essentially dependent on parenteral nutrition (PN), that the obvious drawback of this is liver dysfunction and the presence of infection accounting for

the long-term of central venous catheters [10]. Furthermore, the prevalence of SIBO is associated with the highly significant of central venous catheters by way of the translocation of bacteria and this may depend on the initiation of enteral feeding and weaning off PN [11-14]. Take for example, children with SBS dependence on parenteral nutrition (PN) were a higher percentage of Proteobacteria compared with SBS children weaned off PN. This leads to intestinal dysbiosis in children with SBS related to the long-term PN requirement [14]. The symptoms of IF patients with SIBO exhibit abdominal distension, vomiting, bloating and diarrhea, which sometimes may promote the D-lactic acidosis from the fermentation of the carbohydrates of bacteria to accumulate of D-lactate [15].

The detection of SIBO can be categorized into the direct culture and indirect methods for the aspiration of jejunal fluid and hydrogen or lactulose breath test respectively [16, 17]. Bacterial overgrowth is diagnosed classically to perform the content of bacteria from the jejunal fluid aspiration using culture method, but the negative aspect of this is numerous limitation contained the feasible of specimen contamination from the oropharyngeal organisms while intubation due to the bacterial overgrowth might be unevenly distributed that can be failure with a single time [18]. Accordingly, the indirect method for SIBO had been recommended. The luminal bacteria ferments the carbohydrate such as lactulose, glucose, and xylose that produces hydrogen in exhaled air [18]. Generally, glucose and lactulose hydrogen breath tests have not satisfied to investigate SIBO [18, 19]. Furthermore, many studies have emphasized on feces to explore the microbial throughout the GI tract. This is because both bowel loss and the absorption of the nutrient might be occurred to be clearly conception of SBS. Nearly all microorganisms in the GI tract cannot be detected by culture. For this reason, the gut microbiota composition can be detected deeply with the culture-independent methods, especially 16S rRNA gene sequencing [20].

The antimicrobial agent is the principal weapon for the treatment of SIBO in SBS patients. That is to say, antibiotics can provide against the two aerobic and anaerobic microorganisms. Therefore, metronidazole is an effective treatment for the anaerobic organisms particularly, gram-negative bacteria like *Bacteroides fragilis* and gram-positive bacteria like *Clostridium difficile* [21] and trimethoprim-sulfamethoxazole is a broad-spectrum antibiotic against aerobic microorganisms [22]. Moreover, the treatment of SIBO in children observed with trimethoprim-sulfamethoxazole and metronidazole that were well effective for 20 SIBO children [23]. However, despite the fact

that antibiotics conduct the benefits treatment for the patient, it may result in the risk of multidrug-resistance pathogenic bacteria [24]. In practice, antimicrobial agents can alter not only the target pathogenic bacteria but also the healthy microbiota of the host. It has encountered to affect the long-term gut microbiota changes that connect with clinical diseases [25].

Therefore, prophylactic cyclic antibiotic management can be used to control bacterial overgrowth for SBS patients. Although the alteration of the gut bacterial ecosystem is the aim of therapy, microbiome changes in pediatric patients receiving prophylactic antibiotics have not been studied. Thus, this study aimed to explore the change of the gut microbial composition in pediatric patients with SBS before taking prophylactic antibiotics and after taking each phase of cyclic antibiotics in controlling the bacteria overgrowth of the microbial ecosystem.

Material and Methods

Patients and Sample collection

The study involved fecal specimen collection from pediatric patients with SBS who were receiving nutritional support and monitoring at the pediatric surgical ward, Songklanagarind Hospital, Thailand. As a part of the standard therapeutic program, patients usually receive cyclic alternating antibiotics as gut prophylaxis against bacterial overgrowth. The regimen consisted of oral metronidazole, oral trimethoprim/sulfamethoxazole (Bactrim) and antibiotic-free condition as a 10-day cyclic protocol. Stool specimens on the last scheduled date of each antibiotic regimen and the last date of the antibiotic-free period were collected for gut microbiome study. For stool sample collection, fresh morning stool was collected in 3 sets per patient. Patients were selected in accordance with the inclusion and exclusion criteria. Inclusion criteria for intestinal failure patients was (i) underwent small intestinal resection with the rest of small intestine < 100cm (ii) need to have parenteral nutritional support for longer than 6 months (iii) received prophylactic antibiotics as oral metronidazole, oral trimethoprim/sulfamethoxazole (Bactrim) and antibiotic-free condition alternatively (iv) age newborn to 10 years old. Exclusion criteria were (i) receiving other antibiotics either parenteral or oral route (ii) acute infection or another acute inflammatory process (e.g. upper respiratory tract, pneumonia, urinary tract infection) and (iii) having gastrointestinal bleeding.

After collection and before further analysis, fresh fecal samples were weighed and immediately stored at -20 ^{o}C and -80 ^{o}C.

DNA extraction

The DNA extraction of feces was evaluated using the QIAamp DNA stool mini kit according to the instruction. After that, the purity and integrity of extracted DNA were performed with 260/230 and 260/280 ratios on NanoDrop 2000 (Thermo Fisher Scientific) and 1% agarose gel electrophoresis respectively.

16S rRNA gene Illumina sequencing and Bioinformatics analysis

Good quality and quantity of DNA samples were followed to analyze the Illumina 16S metagenomic sequencing. Firstly, an individual sample was amplified by using the hypervariable region (V3 and V4)of the 341F (5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG 3′) and 806R(5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAA TCC 3′) primers [26]. Briefly, first PCR amplification was demonstrated in the reactions of 25 μL utilized 2.5 μL of template DNA, 1 μM for individual primer, 12.5 μL KAPA HIFI HotStaart ReadyMix (KAPA Biosystems). The cyclic conditions for amplification were in such a way: an initial denaturation for 3 min at 95°C, following by 25 cycles denaturation for 30 sec at 95°C, then annealing for 30 sec at 55°C, after that extension for 30 sec at 72°C, and the final extension for 5min at 72°C. Following this, amplified DNA needed to clean using AMPure XP beads according to the instruction and then checked using a BioAnalyzer 1,000 chip to know the library size for the samples. Afterward, second PCR was performed by attaching Illumina index adapters to the amplified DNA that cyclic conditions were followed for attaching index like 95°C for 3 min, followed by 8 cycles for 30 sec (95°C), 30 sec (55°C),30sec (72°C) and final extension for 5 min (72°C). Thereafter, DNA fragment with adapters checked for purification and quantification used AMPure cleanup and BioAnalyzer 1,000 chip respectively. Ultimately, Sequencing was demonstrated on the flow cell of the Illumina MiSeq platform used reagent kit of MiSeq v3 with paired-end (2x300 bp) run the format sequencing for genomics.

Raw sequence data were demultiplexed and assigned to individual samples according to barcodes that sequence data imported into the Quantitative Insights Into Microbial Ecology (QIIME2 version 2019.7) open-source pipeline [27] utilizing the qiime2-demux plugin. Sequence data were detected in the quality filtering, denoising, merging and removal of chimera by using DADA2 [28]. Subsequently, these sequences were conducted by using MAFFT to be a masked alignment [29] and then QIIME2 using FastTree plugin constructed a phylogenetic tree from a masked alignment [30]. Then, the rarefaction curve was performed at a subsequence depth of 17900 per sample, it is based on the smallest sequence read achieved from each sample. After that, 16s gene sequences were classified into operational taxonomic units OTUs to assign using the 99% of sequences similarity in Greengenes database to obtain the taxonomic group of bacteria composition especially kingdom to species level [31], but species level cannot be identified for the all samples, that is why the operational taxonomic units (OTUs) were analyzed in q2-feature-classifier-plugin with Naive Bayes classifier [32].

Diversity measurement and statistical analysis

Alpha diversity (within the sample) was measured by the Chao 1 index and Ace estimate for the richness of species. Faith's Phylogenetic Diversity (PD) is to determine the biodiversity that sum of phylogenetic branch length connecting between species. Pielou's evenness evaluates how evenly distributed among the species. Shannon's diversity index measures both species evenness and richness of the community using the subsequence depth of rarefaction curve in QIIME2. Beta diversity (between samples) performed using the above rarefaction curve by the Bray-Curtis dissimilarity (BC) that measures the dissimilarity of microbial composition among samples based on abundance into account. Unweighted UniFrac detects the distances of phylogenetic between taxonomy, and or weighted UniFrac distances is to determine the relative abundance into account [33]. The Jaccard index measures the membership of a community that only consists of the shared genera number, not related to their abundance. Beta diversity metrics were visualized by principal coordinate analysis (PCoA) plot utilizing Emperor [34]. For alpha diversity, statistically significant differences in the QIIME2 plugin were calculated using the Kruskal–Wallis test. Statistical significance of p-value is < 0.05. For beta diversity, significance differences in QIIME2 plugin were determined PERMANOVA tests based on 999 permutations.

The relative abundance of microbial composition was showed as means ± standard deviations (SD). The microbial composition was displayed as the bacteria level from phylum to species in the relative abundance of taxonomy.

Results

Illumina Miseq sequencer was processed a total amount of 6,741,196 sequences of paired-end read for 30 samples (Mean = 224706, standard deviation = 336954). A total of 30 samples from under 10-year old pediatric patients prescribing prophylactic cyclic protocol were involved in this study. These were analyzed by bioinformatics analysis. After quality filtering and chimera detection, a total sequence generated 3,188,007 read with an average of 106266 and SD 163248 read per sample (with an average of 106266 ± 163248 sequences per sample) generated totally into 682 OTUs. Sequence read per sample can be seen in the supplementary table (1). The number of OTU for metronidazole, Bactrim and antibiotic-free condition were 182 (mean 18.2, SD 10.4), 263 (mean 26.3, SD 26.6) and 237 (mean 23.7, SD 15.0) respectively and (OTUs can be seen in the supplementary table 2) at 99% sequence similarity against Greengene database. The percentage of OTU annotation were classified from the phylum to the species.

Diversity Analysis

The diversity of microbial composition in the gut was studied according to statistical analysis. The alpha and beta diversity based on the rarefaction curve analysis were calculated with the sampling depth of 17900 sequences per sample. The minimum sampling depth was 17900 reads in this study that was detected to the minimum downstream analysis. The rarefaction curve for observed OTU was increased in antibiotic-free conditions, followed by Bactrim and then Metronidazole. However, the rarefaction curve for Shannon was higher sequence count in Bactrim, followed antibiotic-free and then metronidazole in fig (1A, 1B). The rarefaction curve for alpha diversity can be seen in each sample in the supplementary fig (1). Alpha diversity for gut microbiota was not statistically significant among the three conditions. Although statistic was not

significant, the antibiotic-free condition was substantially increased in the Faith's Phylogenetic Diversity compared to metronidazole (P=0.28) and Bactrim (P=0.40). Moreover, Chao 1 (p= 0.97) and Ace (p= 0.97) indexes were higher in antibiotic-free conditions than metronidazole and Bactrim in Fig (2A, 2B, 2C). Conversely, Pielou's evenness (p= 0.37), and Shannon diversity (p = 0.41) were slightly decreased in antibiotic-free condition compared to metronidazole and Bactrim in Fig (2D, 2E). Alpha diversity for gut microbiota composition was not significantly difference in any index comparison. However, antibiotic-free conditions for gut microbiota consisted of the higher species richness, but species evenness in gut microbiota was minimally different (lower) than antibiotic conditions. Beta diversity distances in gut microbiota of SBS patients were differently observed analyzing Jaccard (p-value=0.999) and Bray-Curtis (p-value=0.978), unweighted and weight Unifrac (p-value =0.122 and p-value=0.321 respectively) distances. Beta diversity can be recognized to distance in each sample and each group in supplementary Fig (2A, 2B). The principal coordinate analysis (PCoA) revealed a significant difference in the gut microbial composition among three conditions. The PCoA plot based on unweighted Unifrac distances of PC1 20.10 % and weighted Unifrac distances of PC1 56.85 % displayed clearly cluster in the microbial composition to each group especially antibiotic-free condition, metronidazole and Bactrim in Fig (3A, 3B). Moreover, these three phases in PCoA contributed to the uniformly assembled using Jaccard in Fig (3C), Bray-Curtis in Fig (3D) (the distance variation of PC1 14.01 % and PC1 33.90 % respectively).

Microbiota Composition

The gut microbiome composition in SBS patients comprised seven phyla constituting Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia, Fusobacteria, and Cyanobacteria. The two phyla of Proteobacteria and Firmicutes were prominently dominated in our study. In the metronidazole condition, Proteobacteria was significantly increased than Firmicutes, with 68.4% against 24.8%. Moreover, more Proteobacteria showed in Bactrim condition than Firmicutes, with 67% as opposed to 25.2%. However, the antibiotic-free condition was exhibited by as many Firmicutes as Proteobacteria, with about 45% in each in Fig (4A, 4B). Besides, the percentage of Bacteroidetes was equivalent to all three conditions, with over 6% in each. The rest of the phyla were less dominant in three conditions especially < 1% in each.

However, Fusobacteria could not be included in Bactrim and Verrucomicrobia only included in the antibiotic-free condition in fig (4). The supplementary table can be seen as the microbiota data for each condition in a table (3).

The relative abundance of bacteria in SBS patients showed the class level of Gammaproteobacteria with 66.7%, 66.2% and 42.9% in metronidazole, Bactrim and antibiotic-free condition respectively. Moreover, Bacilli from the phylum of Firmicutes exhibited 24.5% in antibiotic-free phase, 23.7% in the phase of metronidazole and 16.2% in the phase of Bactrim. The class level of Clostridia had the highest percentage in the antibiotic-free condition, followed by Bactrim and then Metronidazole, with 20.6%, 8.9 %, and 1% respectively. Also, the percentage of Bacteroidia was the same as that of cyclic protocol, with around 6% in each. The antibiotic-free condition was more displayed in the percentage of Betaproteobacteria than metronidazole, with 2.5% and 1.5% respectively, followed by 0.6% in Bactrim condition. Moreover, Alphaproteobacteria was almost absent in three conditions including 0.03% of Metronidazole, 0.11% of Bactrim and 0.05% of antibiotic-free conditions. Actinobacteria was present in the small amount of gut microbiota in SBS patients particularly 0.33% in metronidazole, 0.95% in Bactrim and 0.41% in antibiotic-free conditions. The class level of Chloroplast from Cyanobacteria phylum was rather small in the gut microbiota of SBS patients. Fusobacteriia contained just two phases namely 0.5% in the antibiotic-free condition and 0.04% in metronidazole. Interestingly, Verrucomicrobiae was only present in 1.3% of antibiotic-free conditions.

Considering the Family level, Metronidazole and Bactrim had significantly higher composition of Enterobacteriales than the antibiotic-free condition, with 65% against 42%. Nevertheless, Veillonellaceae was substantially increased in antibiotic-free conditions than in Bactrim, with 15.5% in contrast to 2.3%, followed by 0.3% in metronidazole period. Moreover, antibiotic-free conditions and metronidazole more likely to grow Lactobacillaceae than Bactrim, with 22% as opposed to 13% in Fig (5A, 5B, and 5C). Moreover, Bactrim had marginally higher in Streptococcaceae than in antibiotic-free condition and metronidazole. Neisseriaceae, Clostridiaceae, and Bifidobacteriaceae had extremely small percentages in metronidazole, Bactrim and antibiotic-free conditions, with < 1% in each. An equal percentage of metronidazole and Bactrim conditions revealed in Aeromonadaceae, with < 1% in each. The antibiotic-free condition had 2 times more likely than metronidazole to show in Alcaligenaceae, with 2.4% as opposed to

1.2%. Additionally, antibiotic-free conditions and Bactrim displayed in < 1% of Lachnospiracee in each. Ruminococcaceae was observed in the comparable percentage during antibiotic-free conditions and Bactrim condition, with < 3% in each. More Verrucomicrobiaceae than Fusobacteriaceae revealed just only in antibiotic-free conditions, with 1.3% against 0.5% in Fig (5).

With regards the genera level, the percentage of Escherichia increased in metronidazole and Bactrim conditions compared with the antibiotic-free condition, with 61% in each against 39.7%. In contrast, the relative abundance of genera contained Veillonella was substantially growing in antibiotic-free conditions than metronidazole and Bactrim, with 11.1% as opposed to < 1% in each. Lactobacillus was improved in metronidazole and antibiotic-free condition, with 22% in each than 12.6% of Bactrim condition in Fig (6A and 6D). The same percentage of metronidazole, Bactrim and antibiotic-free conditions reported in the relative abundance of Bacteroides, with around 6% in each. Moreover, Bactrim, antibiotic-free and metronidazole represented 3.1%, 2.7% and 1.2% of Streptococcus, respectively. Proteus and Aeromonas from the class level of Gammaproteobacteria were reported with around 2% and around 1% in each condition, respectively. In the genera level of Klebsiella, there were 2.7% of Bactrim, followed by 1.4% of antibiotic-free and then 0.3% of metronidazole in Fig (6C and 6D). The genus of Morgane and Bifidobacterium were almost scarce in three conditions, with <1% in each. However, Neisseria was only described in 0.4% of metronidazole. Sutterella genus was present in 2.5% of antibiotic-free conditions, followed by 1.2% of metronidazole. Antibiotic-free condition was only reported in 1.3% of Akkermansia, 4.3% of Megasphaera and 0.5% of Fusobacterium. However, the relative abundance of Clostridium from the family level of Peptostreptococcaceae was only demonstrated in 2.0 % of Bactrim. 2.4%, 1.6%, and 0.1% of the genus level of Oscillospira, Megamonas, and Clostridium respectively expressed in the Bactrim phase, while 0.6%, 0.1% and 0.7% of Oscillospira, Megamonas, and Clostridium respectively expressed in the antibiotic-free condition in Fig (6).

In species level, Escherichia coli was strongly grown in metronidazole and Bactrim compared to antibiotic-free condition, with 61% in each against 39.7%. Besides, the relative abundance of Lactobacillus delbrueckii was shown in 7.3%, 7.1% and 6.4% of antibiotic-free conditions, metronidazole, and Bactrim respectively. 8.8% of the antibiotic-free conditions

exhibited in Veillonella dispar. On the other hand, only < 1% of antibiotic conditions showed it. Bacteroides fragilis was approximately a similar percentage in three conditions, with around 6% in each. Lactobacillus helveticus was reported in 12.3% of metronidazole, 9.1% of the antibiotic-free condition and 4.6% of Bactrim. Moreover, Lactobacillus mucosae were performed in 3.2%, 1.2% and 0.6% of antibiotic-free, Metronidazole and Bactrim respectively. There were more antibiotic-free conditions than metronidazole and Bactrim which showed in Veillonella parvula, with 1.9% in contrast to <1% in each. Streptococcus luteciae was demonstrated in 2.4%, 1.9% and 1.0% of Bactrim, antibiotic-free and Metronidazole respectively. Similar proportions of metronidazole, Bactrim and antibiotic-free conditions reported in Lactobacillus reuteri, Lactobacillus salivarius, Morganella morganii, and Bifidobacterium breve, with < 1% in each species of each condition. Clostridium perfringens and Lactobacillus pontis were almost undetectable levels in antibiotic-free conditions and Bactrim. The antibiotic-free condition was only detected in 1.3% of Akkermansia muciniphila.

Discussion

This is the first study of the gut microbiota alteration during administration of the cyclic antibiotic protocol in SBS pediatric patients. In healthy persons, after birth, the component of gut microbiota would stable after three years old. The intestinal microbiota consists of the two prominent phyla of bacteria, *Firmicutes* and *Bacteroidetes* constituted over 90% of the human gut microbiota [35, 36]. However, previous studies and ours have revealed that pediatric SBS patients harbored significant difference in the microbiome composition [14, 37, 38]. Moreover, the consequence of SIBO would be caused by alteration in the ratio and type of gut microbiota [39]. Besides, the treatment of antibiotics can influence the perturbation of gut microbiome using oral or intravenous action. The gut microbiota alteration (perturbation) relies on the classes of antibiotics, dose, and duration as well. Specific antibiotics might affect the respective properties and secretion system leading to the different alterations of the gut microbiota composition [40, 41]. This is clearly illustrated by the fact that vancomycin treatment reported the reduction of microbiome diversity, decreased Firmicutes, and increased proteobacteria. Penicillin treatment also resulted in the drop of Firmicutes, although to a lesser percentage compared with vancomycin[42]. In our study, we observed the alteration of gut microbiota composition during

the cyclic protocol. Rarefaction curves for observed OTU and faith phylogenetic diversity were higher in the antibiotic-free conditions which showed the higher richness of species, whereas the Shannon diversity index in rarefaction was slightly decreased in microbial diversity compared to antibiotic conditions. The effect of this might be a higher level of the commensal bacteria for SBS patients during the cyclic protocol. In practice, Firmicutes phylum had the greater percentage in antibiotic-free conditions compared with the prophylactic phases. However, the phylum of Bacteroidetes had no significant difference among the three conditions. Interestingly, in our study, richness in the family level of Lachnospiraceae and Ruminococcaceae, was observed during the antibiotic-free and Bactrim phase. In addition, Bacteroidceae persisted in all phases of prophylaxis. These commensal anaerobes generate short-chain fatty acid (SCFAs) [43], which could maintain the energy for the host, intestinal epithelial cell nourishment, the absorption of sodium development, regulation of the colonic pH. Furthermore, previous studies suggested that SCFAs play an essential factor in the intestine to prohibit the pro-inflammatory cytokine expression that induces IL-10 production, modulation of T cells (Tregs) activation and alleviate intestinal inflammation [44, 45]. In addition, SCFAs especially constitute propionate, butyrate, and acetate which would be a benefit for epithelial cell proliferation, cell differentiation, and metabolism in the intestine. Among the three groups, butyrate can supply energy for epithelial and promote intestinal barrier function. For butyric acid, it can play the key role of an immunoregulatory molecule for cell cycle inhibition, induction of programmed cell death, and different types of cell differentiation. Recently, propionate and butyrate are detected to the main function of regulatory T cells (Tregs) Foxp3+ production, while Tregs can suppress the main role of inflammatory responses [46]. Butyric acid generates from most bacteria of Firmicutes [47]. Therefore, deficiency of this commensal microbiota can cause to diminish the production of SCFAs and eventually lead to damage to the function of the mucosa, impaired fluid and water absorption, and improved inflammation in the gut [44].

In our study. *Akkermansia muciniphila* belonging to the phylum of Verrucomicrobia represented only in the antibiotic-free conditions. The species has an important function to maintain the intestinal barrier and metabolic homeostatic functions [48]. One clear example of this is that *Akkermansia muciniphila* as a prebiotic was able to develop diet-induced obesity, adipose tissue metabolism and fasting glycemia [49]. When providing this organism that would improve

for treatment of obesity that related metabolic disorders, but the regulation of energy metabolism with this bacterial has not known clearly.

For antibiotic conditions especially metronidazole condition decreased substantially in species richness and microbial diversity compared to Bactrim and antibiotic-free condition. Bactrim condition also decreased the species richness than antibiotic-free conditions, whereas microbial diversity was the highest within three conditions. Even though increasing the bacteria diversity in antibiotic condition particularly in Bactrim, the commensal microbiota in the gut being beneficial for the health was diminished during antibiotic conditions. The reason why the commensal microbiota declined is to compensate for an increase tremendously in the phylum of Proteobacteria in antibiotics conditions. The phylum of Proteobacteria are Gram-negative bacteria produced lipopolysaccharide (LPS), a potency of the anti-inflammatory hepatotoxic compound and commonly present only a small amount around 1%-2% of the gut microbiota. Bacterial species from this phylum is an opportunistic pathogen, the highest abundant species existing was Escherichia coli [50, 51]. Moreover, the family level of Enterobacteriaceae from the class of Gammaproteobactetria includes pathogenic bacteria like Klebsiella species and Escherichia coli that are commonly reported in the tiny number of abundant in a healthy gut, whereas these species are dominant in bacterial overgrowth and intestinal dysbiosis [52, 53]. Here, E. coli was enormously grown in the treatment of antibiotics condition compared to the antibiotic-free phase. For example, E. coli was significantly increased in Streptomycin-treated mice [54]. It is a fact that the treatment of antibiotics also encourages antibiotic-resistant strain development that can contribute to the long-term reservoir of resistance genes in the intestinal microenvironment [55].

The greater amount of Proteobacteria was observed in the gut of human consumed huge amount of fat and carbohydrate, and small amount of fiber [56], chronic malnutrition of children in developing countries [57, 58], and especially adult disease mentions like inflammatory bowel disease [59], type 2 diabetes mellitus [60] and obesity [61]. Hence, the gut microbiome composition is nearly related to enteral feeding. Furthermore, most Proteobacteria species include in SBS patients [14]. The high prevalence of these species contribute to the gut barrier dysfunction with multiple risk factors and the inflammation of the mucosa and as a result of bloodstream infection, translocation of bacteria and prolonged in bowel adaption[11, 62, 63]. Besides, previous studies demonstrated the children with intestinal failure that the outcome of Proteobacteria would

be substantially related to the dependence of PN and liver steatosis [14, 37]. For instance, a prescription of LPS in the animal models leads to hepatic steatosis, and LPS inducing gut microbiota is able to cause Non-alcoholic fatty liver disease (NAFLD) in humans [64].

In our study, the antibiotic condition was revealed a higher prevalence of pathogenic bacteria. Take for example, Broad-spectrum antibiotics that could disturb 30% of the relative abundance of bacteria in the intestinal microbiota accounted for a fall considerably in species richness, evenness and bacteria diversity [65, 66]. Despite the moment of antibiotic treatment has paused, the microbiota composition in the gut was not completely regained to the initial state, but the gut microbiota might report a certain resilience degree. Actually, antibiotic-induced alteration of the intestinal microbiota could persist after prolong time, the length of several months and even years [65-68]. On the other hand, SBS pediatric patients have prescribed the prophylactic cyclic protocol for every ten days in our study. That is why, an antibiotic condition particularly metronidazole and Bactrim has altered the gut microbiota composition especially Proteobacteria remarkedly increased and Firmicutes substantially declined, whereas antibiotic-free condition was the higher proportion of Firmicutes and the reduction of Proteobacteria. Therefore, even though the alteration of gut microbiota composition was not absolutely recovered to a resilient state, the antibiotic-free condition has observed the alteration of beneficial microbiota composition for SBS pediatric patients.

In conclusion, there are conspicuous for the alteration of gut microbiota during the prophylactic antibiotic treatment in our study. Moreover, different types of antibiotics could react to the different effects on gut microbiota in the host. However, in our study, the antibiotic-free condition was dominated in commensal microbiota especially the member of Firmicutes. Moreover, we have studied to better control for clinical strategies development in SBS patients by practicing the prophylactic treatment. For this reason, prophylactic cyclic antibiotic treatment for SBS pediatric patients was effective and controlling the bacterial overgrowth of the microbial ecosystem.

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