Changes in the gastrointestinal microbiota of children with acute lymphoblastic leukaemia and its association with antibiotics in the short term

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Abstract

Purpose. To detect the alteration of gut bacteria in children with ALL and analyse the impact of short-term-use of antibiotics on the changes caused by ALL.

Methodology. We collected faecal samples from both children with ALL and healthy children. According to their medication history with antibiotics, we classified the samples into ALL+ATBx, ALL, CON+ATBx and CON groups. Next-generation sequencing was performed to identify the gut bacteria according to the MiSeq platform. The Shannon index, Simpson index, Chao index and Ace index were used to represent the alpha diversity of gut bacteria. The beta diversity was estimated using the principles of co-ordinate analysis and non-metric multi-dimensional scaling. The taxon composition and presence of biomarkers were then determined through bioinformatics.

Results. With regard to alpha diversity, the Shannon index and Simpson index differed significantly between the ALL and CON groups, as well as the CON+ATBx and CON groups, but not the ALL+ATBx and CON+ATBx groups. With regard to beta diversity, the ALL and CON separated clearly into clusters, as did ALL+ATBx and CON+ATBx. There were differences in composition among the four groups at different taxonomy hierarchies. More bacteria showed an obvious difference between the paired groups ALL and CON than did for the paired groups ALL+ATBx and CON+ATBx. The area under the receiver operating characteristic curves for Bacteroidales and Enterococcaceae used to predict ALL were 0.735 and 0.724, respectively.

Conclusion. ALL induced structural changes of the gut microbiota, with the alpha diversity being significantly weakened by antibiotics, but not beta diversity. Bacteroidales and Enterococcaceae can be referred to as biomarkers for ALL.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is a malignant disease of the bone marrow that is characterized by the overproduction and accumulation of cancerous and immature white blood cells, which are known as lymphoblasts. ALL is the most common type of leukaemia in children, and its incidence rate peaks in children aged from 2–5 years [1]. The incidence rate of ALL is four–five times that of acute myeloid leukaemia and ALL is the leading cause of death in children. An estimated 54 000 cases of ALL are diagnosed in Asia annually, 80 % of which are for children [2]. Although the aetiology and pathogenesis of ALL remain unclear, some environmental risk factors and genetic variables [3] associated with the development and progression of childhood ALL have already been determined. Some evidence has implied a close association between ALL and immune response, and some studies reported a reduction in the antineoplastic immune response in patients with ALL [4]. As a part of the immune system, the digestive tract has 70 % of the immune cells of the entire body. Gut-associated lymphoid tissue (GALT) is an essential part of the immune system, because it regulates systemic immunity [5]. The development of GALT relies on co-growth of the host and symbiotic micro-organisms [6]. In terms of metabolism and epigenetics, the gut bacteria modulate the glycosylation profile [7], which participates in neoplasia [8].
Diseases also affect the composition and function of the gut microbiota. In particular, the intestinal microbiota are affected by many diseases, such as cancer (intestinal tumours and extra-intestinal tumours) [9], metabolic syndrome [10], liver disease [11], allergies [12], Clostridium difficile infection-associated diarrhoea (CDAD) [13], inflammatory bowel disease [14] and graft-versus-host disease [15]. Some researchers have confirmed that antibiotics can reduce the efficacy of antineoplastic treatment by pathogenic Th17 cells [16]. The composition of the gut microbiota and the condition of the host influence each other. Thus, ALL may have a close relationship with gastrointestinal (GI) microbiota.

The human intestine harbours approximately 100 trillion bacteria, which exceeds the amount of human cells by 10-fold [17]. In addition, the microbiome encodes unique genes that are 100-fold greater than those encoded by the genome of its human host [18]. The infant gut microbiota and its corresponding genes (the microbiome) undergo dynamic changes during development, and attain an adult-like microbiome when the child reaches approximately 3 years of age [19]. The difference between the GI microbiota of children with ALL and those of healthy children must be identified to exploit the association between ALL and GI microbiota. The composition of the GI microbiota can be obtained easily by using next-generation sequencing (NGS), which can also elicit some functional diversity. For example, the ratio between Firmicutes and Bacteroidetes (F/B) can reflect the body’s health status and be used to evaluate the homeostasis of the alimentary canal [13].

As is well known, the gut microbiome plays an important role in antitumour immunity [20]. However, most studies focus on digestive system tumours, such as liver cancer and colorectal cancer, and few studies focus on leukaemia. Although the GI microbiota profiles of patients with ALL were verified to change after chemotherapy, and the composition and alpha diversity can distinguish ALL patients from controls without regard to antibiotics [21], the changes in the beta diversity and composition in pretreated ALL children remain unclear. With the high morbidity of ALL, determining the relationship between ALL and the GI microbiome accurately and comprehensively is necessary. To this end, we experimented on the faecal microbiota of children with ALL.

Patients with ALL have generally been treated with antibiotics before diagnosis with ALL to treat infections, which are a major symptom of ALL. Antibiotic administration affects the GI microbiota significantly. Thus, the ALL and non-ALL groups were classified into two groups to avoid the bias caused by antibiotics. One group comprised the patients administered with antibiotics and the other group comprised those that were not. The rationale of this study is to analyse the changes in the GI microbiome in children with ALL through 16S ribosomal-RNA gene sequencing and elucidate how ALL influences the GI microbiome.

**METHODS**

**Subjects recruitment and sample collection**

This study cohort involved 63 participants; 30 patients had ALL and 33 were healthy subjects and were used as controls. The 30 patients were diagnosed with ALL from December 2014 to November 2015, and they were diagnosed according to the morphological, immunological, cytogenetic and molecular (MICM) standards. Of the 30 patients, 2 were diagnosed with T-cell acute lymphoblastic leukaemia, and 28 were diagnosed with B-cell acute lymphoblastic leukaemia. The 33 healthy children were selected from different communities in Jinan. Each participant had an appropriate body mass index (BMI) and was not administered with probiotics, prebiotics, or synbiotics for 3 months before the present study. There were no significant differences between the daily dietary habits of the healthy children and the ALL children (Tables S1 and S2, available in the online Supplementary Material). In addition, they had not suffered from diarrhoea for at least 1 month before the faecal samples were collected. Each of the two groups was divided into two subgroups according to their use of antibiotics, such as penicillin or cephalosporin, before sample collection. The demographics of the participants are shown in Table 1. Every child in the ALL+ATBx and CON+ATBx groups took cephalosporin orally twice a day for 3–5 days, and intravenous penicillin or cephalosporin for 7–10 days. All of the children who took antibiotics were mildly-to-moderately infected, and the dosage per kilogram and frequency of antibiotics use were similar. There were 13 cases of bronchitis, 5 cases of bronchopneumonia, 1 case of arthritis and 1 case of fever of undetermined origin in the ALL+ATBx group.

The sampling protocol for the present study was approved by the Ethics Committee of Qilu Hospital (Shandong, PR China) and informed consent was obtained from the parents or guardians of the children. The faecal samples were collected from the patients at diagnosis, before chemotherapy was administered. We collected at least 2 grams of fresh faecal sample from each participant using sterilized micro-spatulas. The collected faecal samples were placed into separate sterilized cryogenic vials. The faecal samples were frozen in liquid nitrogen within 30 min after preparation and then stored at −80°C for further analysis.

**DNA extraction, bacterial 16S rRNA gene amplification and next-generation sequencing**

After genomic DNAs were extracted from the faecal samples using the EZNA stool DNA kit (OMEGA, Norcross, GA, USA), the bacterial genomic DNA was amplified in triplicate using the 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers with barcodes specific to the V3–V4 hypervariable regions of the 16S rRNA gene. PCR was then performed with the ABI GeneAmp 9700 using TransGen AP221-02: TransStart FastPfu DNA polymerase. The amplicons were separated by 1% agarose gel electrophoresis and then
purified using the AxyPrep DNA PCR purification kit (Axygen, China). The purified amplicons were then quantified with a QuantiFluor-ST handheld fluorometer with UV/blue channels (Promega Corporation, Madison, WI, USA). The quantified amplicons were sequenced on an Illumina MiSeq platform at the Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, PR China).

Bioinformatics and statistical analysis

The primer and adapter sequence of the 3’ end of each read was trimmed within the parameter ‘maximum number of mismatches = 3’ using Trimmomatic (http://www.usadellab.org/cms/index.php?page=trimmomatic). The base quality of the test window that was shifted at one-base pair step width was set at 50 bp in length. When the mean quality in the window was lower than 20, the preceding sequence was intercepted from this position. The paired-end reads were merged with FLASH (http://ccb.jhu.edu/software/FLASH) when an overlap with a minimum setting of 10 bp was intercepted between them. The valid reads were clustered into operational taxonomic units (OTUs) with 97% similarity using Usearch (version 7.1, http://drive5.com/uparse/). The OTUs used for community richness (ACE and Chao1), community alpha diversity (Shannon and Simpson), Good’s coverage and rarefaction curve were analysed by Mothur version version 1.30.1 [22]. The taxonomic classifications of these OTU sequences at the genus level were determined with RDP classifier [23] (http://sourceforge.net/projects/rdp-classifier/) based on QIIME (http://qiime.org/scripts/assign_taxonomy.html) at a 70% confidence threshold. The Bray–Curtis similarity index represented in the heatmap [24] was used to compare the samples according to the abundance of OTUs among them. An unweighted pair-group method with arithmetic means (UPGMA) clustering was performed using QIIME software to interpret the distance matrix using average linkage [25].

Statistical analyses

The independent t-test and Fisher’s exact text were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) to compare the microbiome features of the sample groups and the distribution of children using different antibiotics, respectively. The characterization of the micro-organisms for differentiation of the faecal microbiota specific to different toxigenic types was performed using linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/) for biomarker discovery [26]. This procedure emphasized both statistical significance and biological relevance. A significance alpha of 0.05 and an effect size threshold of 2 were used for all the biomarkers discussed in this study. All tests for significance were two-sided, and a P value of 0.05 was considered to be statistically significant. The receiver operating characteristic (ROC) curve for the bacteria and ALL was analysed using SPSS 17.0.

RESULTS

Cohort description and sequencing data

The study cohort is described in Table 1. The mean age and BMI differences between each group were not significant according to Student’s t-test (P>0.05). The distribution of children using different antibiotics in the ALL+ATBx and CON+ATBx groups is shown in Table S3. There is no significant difference in the proportion of penicillin or cephalosporin in the different groups when analysed by Fisher’s exact text (P>0.05). A total of 63 faecal samples were collected and used for next-generation sequencing. The next-generation sequencing studies provided 2,759,969 valid sequences, with an average read length of 436.78 base pairs. The flattening trend for the rarefaction and Shannon–Wiener curves of all the samples reflected the sufficiency of the sequencing quantity. The Good’s coverage was above 99.7%, indicating the sufficiency of the sequencing depth.

Patients with ALL can be distinguished from controls without the administration of antibiotics in terms of alpha diversity

To assess the diversity of the GI bacteria, we grouped the ALL, CON, ALL+ATBx and CON+ATBx groups into the following pairs: ALL+ATBx and ALL, ALL+ATBx and CON+ATBx, ALL and CON, and CON+ATBx and CON. By using an independent variable t-test, we found that the mean of the
Shannon index (Fig. 1a) in ALL was significantly lower than that in CON (P<0.05). By contrast, the Simpson index (Fig. 1b) in ALL (P<0.05) was higher than that in CON, indicating that the species diversity of the GI bacteria in children with ALL was lower than that of the children without ALL when they were not administered with antibiotics, as the higher the Shannon index, the higher the species diversity, whereas the higher the Simpson index, the lower the species diversity [27]. Similarly, the two indices of the gut bacteria of CON+ATBx were different from those of CON, indicating the decline in diversity caused by antibiotics. This result was consistent with that from a previous work [28]. However, there was no statistical significance among the means of the diversity indices of the ALL+ATBx and ALL group, or the ALL+ATBx and CON+ATBx group (P>0.05). Thus, ALL and the administration of antibiotics reduced the diversity of GI microbiota in children. The ALL+ATBx group showed significantly reduced diversity compared with that of the CON group (P<0.01) (Fig. 1a, b). In addition, the species richness estimated by the Chao1 and Ace indices (data not shown) of the gut bacteria among the pair groups showed no apparent difference (P>0.05).

ALL led to the alteration of beta diversity regardless of whether antibiotics were administered

Beta diversity analysis was performed to measure the extent of the similarities and differences among independent microbial communities. According to the Pcoa (Fig. 2b) and NMDS (Fig. 2e) analyses, the ALL and CON, as well as the paired groups ALL+ATBx and CON+ATBx (Fig. 2c, f), were separated into distinct clusters, despite apparent inter-individual variations. Nonetheless, these distinct clusters were not observed in the other two paired groups. The administration of antibiotics thus had no evident influence on the variation of the beta diversity caused by ALL. In addition, the samples in the CON group were mostly concentrated among the four groups (Fig. 2a, d). In summary, both the alpha and beta diversities of the gut bacteria were disrupted by ALL, but the disruption was weakened by the antibiotics for alpha diversity.

A dendrogram (Fig. S1) was obtained through hierarchical clustering analysis, based on the beta diversity using the UniFrac unweighted distance. The dendrogram demonstrated the phylogenetic distance among the samples and indicated that the GI microbiota of children within the ALL and ALL+ATBx groups were phylogenetically near, whereas those of the children in the other two groups without ALL were phylogenetically distant.

**ALL-associated compositional changes in the GI microbiota**

The taxonomy of the GI microbiota was assessed by a taxon-dependent analysis using the RDP classifier. Overall, 11 phyla, 20 classes, 32 orders, 61 families and 197 genera were detected in the GI microbiota of the totality of faecal samples. Hclust bar (Fig. 3a) and heatmaps (Fig. S2a) showed that, at the phylum level, the most predominant phyla in children were *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*, which are also predominant in the GI microbiota composition in adults [29]. Consistent with the evidence obtained by a previous study [13], the majority of the genera had low abundance in the GI microbiota samples at increased sequencing depth. Of the genera found in the GI microbiota, 17 and 14 abundant genera (mean >1 % of the total DNA sequences) were detected in the ALL+ATBx and ALL samples, respectively. Meanwhile, 18 and 20 abundant genera were found in CON+ATBx and CON samples, respectively. At this level, *Faecalibacterium* and *Bacteroides* were prevalent in the ALL, CON+ATBx and CON groups. However, ALL+ATBx had abundant *Megamonas*, which is under the phylum *Firmicutes*. *Megamonas spp.*, present in the faecal samples in the ALL+ATBx group, was more abundant than in the CON+ATBx and CON groups (P<0.05) (Fig. 3b). This difference was observed in the faecal samples of obese patients [30] and those with

![Fig. 1. Index for the assessment of the alpha diversity of the GI microbiota. The (a) Shannon and (b) Simpson indices were used to estimate the diversity of the faecal microbiota. * indicates P<0.05; ** indicates P<0.01.](image-url)
Fig. 2. Beta diversity graph. GI microbiota from children with ALL and healthy controls were distinguished by PcoA analysis (a–c) and NMDS analysis (d–f). All results indicated that the beta diversity of the GI microbiota was altered by ALL with or without the administration of antibiotics.
CON1
CON19
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ALL23
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ALL2
ALL26

(a) UPGMA clustering of samples on weighted UniFrac revealed the composition of the GI microbiota and the phylogenetical relationships among the samples. The abundance of Megamonas (b) and Blautia (c) varied among the four groups. * indicates P<0.05.

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(b) Relative abundance of Megamonas
(c) Relative abundance of Blautia
HIV-1 infection [31]. The abundance of Blautia in the ALL +ATBx group was lower than that in the ALL and CON +ATBx groups ($P<0.05$), and they were also scarce in the ALL group compared with the CON group ($P<0.05$) (Fig. 3c). The heatmaps in Fig. S2 also show the correlations among the groups at the phylum and genus levels, and highlight the apparent variations according to the extent of ALL or the effect of antibiotics, coupled with clustering analysis.

As an indicator of dysbiosis in the GI microbiota in several diseases [11, 32], the F/B ratios of the four paired groups were compared with one another. In the CON+ATBx and CON paired group, the F/B ratio was significantly decreased in the CON+ATBx group ($P<0.05$), and was approximately one-third of that in the CON group (Fig. 4), indicating the dysbiosis caused by antibiotics. This result was consistent with the results of previous experiments on humans [28] and animals [33]. However, the variations among the other three pairs were not statistically significant ($P>0.05$). Meanwhile, no difference was observed between the abundances of Actinobacteria and Proteobacteria.

**Impacts of ALL on GI microbiota when the interference of antibiotics is excised**

The phylogeny and microbiota composition in the ALL group were compared with those in the CON group. To identify the specific bacteria taxa associated with ALL, we compared the GI microbiota in children with ALL without an antibiotic medication history (group ALL) with those in healthy controls (group CON) using LEfSe. Fig. 5a shows the cladogram that represents the phylogenetic structure of the GI microbiota and their predominant bacteria. The cladogram showed an apparent difference between the GI microbiota of the two groups. Fig. 5b shows the LDA scores of these bacteria. Bacteria in different taxonomic levels that show a great difference and belong to the predominant phyla Firmicutes and Bacteroidetes can be used as biomarkers to distinguish ALL when a child is free from antibiotics. With the significant difference of relative abundance between the two groups, Bacteroidales and Enterococccaeae can be used as a microbial marker for diagnosing ALL when a child is free from antibiotics (the areas under the ROC curves were 0.735 and 0.724, respectively) (Figs 5c and 6a). Furthermore, only a few bacteria signatures in the GI microbiota were observed in the two groups in which antibiotics had been administered (Fig. 5a, e). The LDA scores of these groups were lower (Fig. 5b, f). The area under the ROC curve (AUC) for Enterococcceaeae to predict ALL was 0.694 (Fig. 6b).

The bacteria under Firmicutes were significantly less abundant in the GI microbiota of children with ALL compared to those in healthy controls, whereas the bacteria under Bacteroidetes were apparently more abundant in children with ALL ($P<0.05$, Fig. 7a) than in those in healthy controls. Similarly, Figs 7b–d and S3 represent the relative abundance of bacteria occupying the main part of the community in different taxonomy hierarchies. Bacteroidia and Bacteroidetes were more abundant in children with ALL ($P<0.05$) at the class and order levels, separately. Clostridia and Erysipelotrichaceae, Clostridiales and Erysipelotrichales, Lachnospiraceae and Erysipelotrichaceae, and Blautia were less abundant in children with ALL ($P<0.05$) at the class, order, family and genus level, separately.

**DISCUSSION**

With the advent of NGS, an increasing number of diseases are being analysed with regard to their microbiotic aspect [9–15]. Furthermore, the important role of microbiota in the development and function of the immune system is being considered [4–6, 20]. As an essential function of the immune system, immunological surveillance plays a vital role in antitumour immunity. Dysbiosis is a condition that is characterized by microbial imbalances, and it exhibits changes in the microbiota composition [34]. This condition causes dysregulated immune functions. When dysbiosis occurs in the GI tract, the respiratory tract, the genitourinary tract and the skin, a cascade of events is initiated that eventually leads to malignant growth [9]. In the present study, the application of NGS techniques through sequencing of the 16S rRNA gene facilitated the determination of the relationships among the intestinal microbiota in patients with ALL at the molecular level. The BMIs of the enrolled children were within the range of normal values and without significant differences between the two groups, so dysbiosis caused by obesity or malnutrition could be disregarded. With the high Good’s coverage values, which were all $>99.7\%$, the identified sequences represented the majority of the bacterial sequence, and therefore were representative of the GI microbiota of the children.

In ecology, alpha diversity describes the richness and equitability of within-habitat or within-sample species in our experiment, and beta diversity is the extent of the differentiation of communities along habitat gradients [35]. Our results showed that the species diversity of the GI microbiota of children with ALL in the alpha diversity category

![Fig. 4](image-url) No significant difference was observed between the F/B ratios of children with ALL and healthy children. However, antibiotics decreased the F/B ratios in healthy children. * indicates $P<0.05$. 

*Fig. 4* The F/B ratios of children with ALL and healthy children.
Fig. 5. LEfSe identifying the most differentially abundant taxon in healthy controls and children with ALL who have not had antibiotics administered. The taxonomic cladogram was obtained through LEfSe analysis on the 16S sequences. Red: ALL-enriched taxa. Green: taxa enriched in healthy controls. The brightness of each dot is proportional to its effect size (a). Healthy control-enriched taxa are indicated with a positive LDA score (green), and taxa enriched in ALL have a negative score (red). Only the taxa that satisfy the LDA significant threshold of 2 are shown (b). Similarly, (e) and (f) identify the most differentially abundant taxon among the taxa of the healthy controls and children with ALL under the administration of antibiotics. The receiver operating characteristic (ROC) curves for Bacteroidales (c) and Firmicutes (d) used to predict ALL.
was lower than that of healthy controls without interference from antibiotics. However, the application of antibiotics decreased the diversity of the GI microbiota in healthy children. With regard to beta diversity, the samples from children with ALL diverged from the controls, regardless of antibiotic use. The interference with the GI microbiota caused by antibiotics has been established. Similarly, ALL disrupted the diversity of GI microbiota in this manner. Thus, the changes in beta diversity caused by ALL cannot be eliminated or neutralized by antibiotics.

Firmicutes and Bacteroidetes account for the majority of the GI microbiota in humans [18]. In the present study, the abundance of Firmicutes decreased, and the abundance of Bacteroidetes increased, in children with ALL and without antibiotic influence, which agrees with Rajagopala’s results [21], indicating that ALL induces dysbiosis. Dysbiosis is indicated by a decrease in Bacteroidetes in adult survivors of childhood ALL [36]. With regard to dysbiosis, the F/B ratio can be used as an evaluating indicator. The F/B shows a significant correlation with the composition of the GI microbiota. In previous studies, the F/B decreased under the short-
term administration of antibiotics to human adults and mice [28, 33]. Our study confirmed this finding for children.

To investigate the functional role of the gut microbiota in the four groups, we used the eggNOG database and the KEGG database by PICRUSt, and found that a similar trend was shown in different metabolic pathways (Figs S4 and S5). We inferred from this analysis that antibiotics, as well as ALL, can reduce the metabolic level in different pathways, while they both supplement each other. Some bacteria influenced by ALL or co-influenced by ALL and antibiotics have different functions in metabolism. *Megamonas*, which is correlated with the systemic inflammatory cytokines IL-6 reported in a previous study [31], was quite abundant in children with ALL and children who had received antibiotic treatment <1 month before sample collection (group ALL + ATBx). By contrast, the abundance of *Megamonas* in the other groups did not differ. Presumably, ALL, together with antibiotics, can stimulate inflammation. *Blautia* are potentially beneficial bacteria that can produce acetate, ethanol, hydrogen, lactate, or succinate as end products during glucose fermentation [37]. It was scarce in children with ALL, regardless of antibiotic use. Jenq RR et al. [38] found that *Blautia* is associated with reduced lethal graft-versus-host disease (GVHD), suggesting a link with immune suppression. A subtype species *Blautia obeum* plays an important role in the recovery process from Vibrio cholerae infection [39] and microbiota maturation in children. Furthermore, *Blautia* is associated with anti-inflammatory reactions [38]. ALL alone can decrease the abundance of *Blautia*, without coaction of antibiotics. The dysbiosis caused by ALL can lead to inflammation, directly or indirectly through disrupted anti-inflammation.

In our results, *Bacteroidetes, Bacteroidia, Bacteroidales, Porphyromonadaceae* and *Enterococcaceae* were effective in distinguishing ALL children from controls. Among the microbiological markers of ALL discovered by LEfSe, *Bacteroidales* and *Enterococcaceae* played a vital role because they had a high LDA score and AUC of ROC. *Bacteroidales* (as well as *Bacteroidetes* and *Bacteroidia*) plays a dominant role in the fermentation of complex carbohydrates [40] that provide nutrition and immunomodulation [41] to the host. *Bacteroidales* are enriched in many pathogenic settings, such as hepatitis C [42], colitis [43] and colorectal cancer [44], and in patients after cholecystectomy [45], and revealed the link between GI microbiota and immunodeficiency or malnutrition. Moreover, *Clostridia*, in accord with the quantity of short-chain fatty acids (SCFAs), such as butyric acid [13], are deficient in the ALL group. *Lachnospiraceae*, as a subtype of *Clostridia*, also decreased considerably. SCFAs may enhance colonization resistance and suppress pathogen growth by lowering the pH and redox potential (eH) in the intestinal lumen [46], and thus the decrease in *Clostridia* may result decreased resistance. Although *Firmicutes* was predominant in healthy controls, *Enterococcaceae*, a type of lactic acid-producing bacteria belonging to *Firmicutes*, increased in children with ALL.

Accumulation of lactic acid can increase the permeability of the intestinal mucosa by endotoxaemia, and is closely associated with ulcerative colitis and gut resection, as indicated in the previous studies [47].

In our study, some problems remained unsolved and several limitations were present. First, the bacterial composition diversity was not determined accurately because of the limited sample size and large age span. Thus, a large sample size and analysis according to different age groups are required to amplify the differences and increase the accuracy. Second, this study only demonstrated a single effect of ALL on the GI microbiota. The association between ALL and GI microbiota is mutual. Therefore, animal experiments with faecal microbiota transplantation (FMT) from ALL mice to germ-free mice or those treated with wide-spectrum antibiotics must be performed to illustrate the influence of disturbed gut bacteria on the effects of ALL. Third, we only revealed a phenomenon with a superficial explanation, so molecular studies on cytokines, for example, must be performed.

**Conclusion**

ALL induced dysbiosis by changing the diversity and composition of GI microbiota. Antibiotics weakened the changes in alpha diversity and decreased the LDA score of some bacteria, but had little effect on beta diversity. *Bacteroidales* and *Enterococcaceae* could be considered to be biomarkers of ALL.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

The present study was approved by the Ethics Committee of Qilu Hospital (Shandong, China) and informed consent was obtained from the parents or guardians of the children.

**References**


