Pediatrics & Neonatology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Pediatrics & Neonatology

journal homepage: www.pediatr-neonatol.com

Investigation of gut microbiota diversity according to infectious agent in pediatric infectious acute gastroenteritis in a Korean university hospital

You Ie Kim [a], Sang Yong Kim [a], Seungok Lee [b,*], Myungshin Kim [c], Woo Jin Kim [d]

[a] Department of Pediatrics, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[b] Department of Laboratory Medicine, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[c] Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[d] EONE Laboratories, Incheon, Republic of Korea

ARTICLE INFO

Keywords:
16s rRNA sequencing
Acute gastroenteritis
Gut
Infection
Microbiome
Pediatric

ABSTRACT

Background: Acute gastroenteritis (AGE) is a common cause of pediatric morbidity and mortality worldwide. AGE can cause an imbalance in the intestinal microbiota. This study aimed to investigate the diversity of the gut microbiome in Korean children hospitalized for infectious AGE at a university hospital.
Methods: A total of 23 stool samples from patients aged 5 months to 11 years with AGE were analyzed. Thirteen convalescent stool samples were collected 1 month after discharge. Multiplex polymerase chain reaction (PCR) for the five viruses and 16 bacteria-specific AGE pathogens (PowerChek Multiplex Real time PCR Kit, Seoul, Korea), and 16s rRNA sequencing (Illumina MiSeq Sequencing system, Illumina, USA) were performed.
Results: According to the results of multiplex PCR for causative pathogens, the microbiome taxonomic profile (MTP) of the gut microbiome in three groups of AGE, norovirus AGE (n = 11), *Campylobacter* AGE (n = 7) and *Salmonella* AGE (n = 5) was compared. The phylum Actinobacteria was significantly more abundant in the norovirus AGE ($P = 0.011$), whereas the phylum Proteobacteria was significantly more abundant in *Salmonella* AGE ($P = 0.012$). Alpha diversity, which indicates species richness and diversity, showed no statistical differences. However, beta diversity, representing the similarity in MTP between norovirus, *Campylobacter*, and *Salmonella* AGE, was significantly different ($P = 0.007$). In convalescence, compared with their corresponding AGE samples, the phylum Firmicutes; and the lower taxa Christensenellaceae ($P = 0.0152$) and Lachnospiraceae ($P = 0.0327$) were significantly increased.
Conclusions: In pediatric AGE, the type of infectious agent can affect the diversity and dominance of gut microbiota in pediatric patients. Furthermore, healthy gut bacteria increased during the period of 1 month after infection, allowing a return to a healthy state without causing long-term dysbiosis.

1. Introduction

The human gut microbiota plays an important role in childhood development, maintaining health by producing many nutrients such as short-chain fatty acids and amino acids, maturation of the immune system, and protection of the host from pathogen invasion [1–3]. Intestinal microorganisms are found in the feces of newborns, and the composition of the microbiota varies according to factors such as delivery type, feeding, and diet. At approximately 3 years of age, the gut microbial composition of children changes to a pattern similar to that of adults [1–7].

Acute gastroenteritis (AGE) is a common cause of morbidity and mortality in children worldwide and the fifth leading cause of death, particularly in children under 5 years of age [8,9]. According to studies on pediatric AGE in Koreans, the detection rate of enteric virus is 20–30%, and norovirus is increasing after the introduction of rotavirus vaccination, especially in children under 24 months of age [10–12]. The detection rate of enteric bacterial pathogens is 3–20%, *Escherichia coli* and *Salmonella* spp. are common, and *Campylobacter* spp. are increasing [10–12].

Infectious AGE causes an imbalance in the intestinal microbiota, resulting in dysbiosis [13–18] Dysbiosis is an imbalance in the diversity of the gut microbiota that can lead to altered metabolite profiles and contribute to chronic diseases such as inflammatory bowel disease,

* Corresponding author.
E-mail address: lsok@catholic.ac.kr (S. Lee).

https://doi.org/10.1016/j.pedneo.2024.01.005
Received 10 May 2023; Received in revised form 19 December 2023; Accepted 26 January 2024
Available online 6 March 2024

irritable bowel syndrome, metabolic disorders, and neurodevelopmental disorders [2,19–23]. Previous studies have reported that the diversity of the gut microbiome of children with AGE differs from that of healthy children, [17,18] and is influenced by the type of infectious agent [14–18].

This study was conducted to investigate the diversity of the gut microbiome according to the type of infectious agent in children hospitalized with infectious AGE and to examine changes in the gut microbiota during the acute and convalescent phases.

2. Materials and methods

2.1. Study design and patient enrollment

This study was approved by the local institutional review board (number: OC19TESI0053), and informed consent was obtained from the patients' parents or guardians. Hospitalized patients presenting with sign of AGE, including vomiting, diarrhea, fever and dehydration within 1 week were enrolled at the university hospital from June 2019 to May 2022. Patients with underlying diseases requiring medication, a history of previous abdominal surgery, or a recent history of hospitalization or antibiotic use within the past month were excluded.

Stool specimens from these patients were analyzed to identify the pathogen causing AGE. On hospitalization, acute phase specimens were collected, routine bacterial culture and multiple polymerase chain reaction (PCR) tests for pathogens causing AGE were performed. The leftover specimens were used for microbiome analysis. Convalescent specimens were collected 1 month after discharge for convalescent microbiome analysis. The patients' medical records were reviewed for clinical information including age, sex, diarrhea, vomiting, fever, and dehydration, and laboratory findings and were divided into three groups: bacterial AGE, viral AGE, and convalescent groups. The microbiome analysis results were first compared in three groups; second, the diversity in gut microbiome according to pathogens was examined, and changes in major taxa of enteric bacteria in the acute phase and convalescent samples were investigated.

2.2. Multiplex PCR for specific AGE pathogens

The nucleic acid was extracted using TANBead Nucleic Acid Extraction kit (Taiwan Advanced Nanotech Inc., Taoyuan, Taiwan, [R.O.C.]) for acute phase stool. A multiplex PCR for the five viruses and 16 bacteria causing AGE were performed according to the manufacturer's instructions (PowerChek Adeno/Astro/Rotavirus Multiplex Real time PCR Kit, PowerChek Norovirus GI/GII Multiplex Real time PCR Kit, and PowerChek 19 Pathogen Multiplex Real–time PCR Kit, KOGENEBIOTECH Co Ltd, Seoul, Korea).

2.3. Microbial DNA extraction and 16s rRNA sequencing

The collected samples were stored at −70 °C and thawed at room temperature for 1 h before use. Microbial genomic DNA was extracted from the stool samples. PCR amplification using fusion primers targeting the V3 to V4 regions of the 16s rRNA gene was performed as previously described [24]. The amplified products were purified, and the quality of the products was assessed using a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) with a DNA 7500 chip. Mixed amplicons were pooled and sequencing was carried out at CJ Bioscience, Inc. (Seoul, Korea) using the Illumina MiSeq Sequencing system (Illumina, USA).

2.4. Bioinformatics analyses

Bioinformatics analyses were performed using the EzBioCloud App (CJ Bioscience, Inc., Seoul, Korea) according to a previous study [24]. Alpha diversity was calculated using the Abundance-based Coverage Estimator (ACE) index for species richness and Shannon index for species diversity and evenness. Beta diversity distances were calculated to visualize the taxonomic differences between samples by principal coordinate analysis (PCoA) using the Jensen-Shannon method. Beta set-significance analysis between the two MTP sets was performed using permutational multivariate analysis of variance (PERMANOVA) test. Taxonomic biomarkers, meaning differential taxa of microbial communities between the three groups, were analyzed using linear discriminant analysis effect size (LEfSe) and are displayed as cladograms [25].

2.5. Statistical analysis

Continuous variables were analyzed using Fisher's exact test. Nonparametric tests (Kruskal-Wallis test for more than two groups and Mann–Whitney *U* test for two groups) were used to compare the MTP results. The Wilcoxon rank-sum test was used to compare the gut taxa selected from the individual AGE and paired convalescent samples. MedCalc version 20.118 (MedCalc Software, Ostend, Belgium) was used for statistical analysis. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Patient characteristics

A total of 23 stool specimens from patients with AGE aged between 5 months and 11 years were analyzed. The causative pathogens of AGE, bacterial AGE (n = 12) and viral AGE (n = 11) were grouped based on the multiplex PCR results. The causative bacteria included *Campylobacter jejuni* (n = 6), *Salmonella* spp. (n = 5), *Campylobacter coli* (n = 3), and *Clostridium perfringens* (n = 3). All the viral AGE were infected with norovirus GII (n = 11). In the bacterial AGE group, there were nine cases (75%) of single infection, two cases (17%) of mixed infections with *C. jejuni*, *C. coli* and *C. perfringens,* and one case (8%) of mixed infection with *C. coli* and *C. perfringens*.

In addition, 13 convalescent specimens were obtained from 23 enrolled patients 1 month after discharge. Therefore, the results of the microbiome analysis were divided into bacterial AGE, viral AGE, and convalescent groups. Baseline characteristics and clinical manifestations of the three groups are summarized in Table 1. No enrolled patients received antibiotics. Patients with bacterial AGE were significantly older than those with viral AGE (median [range]: 5.5 years [2.5–11.0 years] vs. 1.2 years [0.42–7.0 years], $P < 0.001$), with no differences by sex, length of hospital stay, or clinical manifestations. Most of the patients with bacterial AGE (11 of 12 patients) had fever and diarrhea, whereas all patients with the viral AGE had vomiting and dehydration. None of the enrolled patients had sepsis or bacteremia. The platelet count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) level differed significantly between the bacterial AGE and viral AGE groups.

3.2. MTP differences in gut of pediatric patients with bacterial AGE, viral AGE, and convalescent groups

The MTP at the bacterial AGE (n = 12), viral AGE (n = 11), and convalescence (n = 13) are shown in Fig. 1A. The phylum Actinobacteria was significantly more abundant in viral AGE (median % abundance, 0.5%, 10.5%, and 4.2% in bacterial AGE, viral AGE, and convalescent groups, respectively, $P = 0.003$); the phylum Proteobacteria was significantly more abundant in bacterial AGE (28.1%, 7.1%, and 1.6% in bacterial AGE, viral AGE, and convalescent groups, respectively, $P = 0.003$); and the phylum Firmicutes was most abundant in convalescence (31.8%, 29.4%, and 49.1% in bacterial AGE, viral AGE, and convalescent groups, respectively, $P = 0.046$). In the bacterial AGE, viral AGE, and convalescent groups, neither the alpha diversity of the ACE index, which indicates species richness (median, 210, 240, and 197, respectively, $P = 0.787$), nor the Shannon index, which indicates species diversity (median, 2.125, 2.273, and 2.466, respectively, $P = 0.329$),

Table 1
Basal characteristics and clinical manifestations.

Variable	Bacterial AGE (n = 12)	Viral AGE (n = 11)	Convalescence (n = 13)	*P* value[a]
Male sex, n (%)	8 (67)	7 (64)	7 (54)	0.794
Age, years, median (range)	5.5 (2.5–11.0)	1.2 (0.42–7.0)	3.0 (0.4–8.0)	<0.001
Length of hospital stay (days), median (range)	4 (1–9)	3 (1–5)	–	0.452
Clinical manifestations, n (%)				
Diarrhea	11 (92)	7 (64)	–	0.213
Vomiting	4 (33)	11 (100)	–	0.064
Fever	11 (92)	8 (73)	–	0.476
Dehydration	8 (67)	11 (100)	–	0.690
Laboratory finding [median (range)]				
WBC count(/μL)	7,450 (4,700–19,060)	10,570 (4,880–19,890)	–	0.151
ANC (/μL)	5,326 (2,906–9,682)	6,585 (2,130–11,835)	–	0.880
Hemoglobin (g/dL)	13.0 (11.6–13.7)	12.3 (11.0–14.0)		0.091
Platelet count (/μL)	270,000 (171,000–474,000)	383,000 (220,000–557,000)		0.027
ESR (mm/h)	38 (2–88)	11 (2–50)		0.007
CRP (mg/L)	68.0 (0.1–185.2)	1.73 (0.1–63.0)		0.019
Pathogens detected				
Single	9 (75%)	11 (100%)		
Multiple	3 (25%)			
Pathogen (n)	*Campylobacter jejuni* (n = 6) *Salmonella* spp. (n = 5) *Campylobacter coli* (n = 3) *Clostidium perfringens* (n = 3)	Norovirus GII (n = 6)		

AGE, acute gastroenteritis; ANC, absolute neutrophil count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell.
[a] *P* values were calculated using non-parametric tests between bacterial AGE and viral AGE.

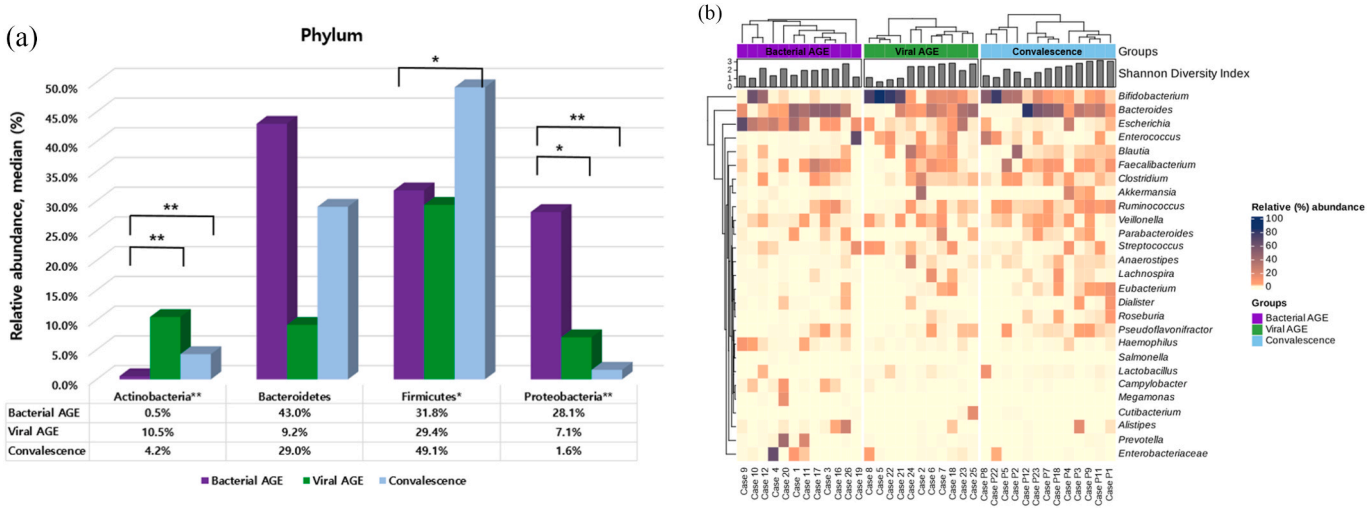

Fig. 1. Comparison of gut microbiome taxonomic composition (MTP) in pediatric patients with bacterial AGE (n = 12), viral AGE (n = 11), and convalescence (n = 13). (A) Relative abundance, median (%, y-axis) of gut MTP at phylum level in three groups (x-axis) (*$P < 0.05$ and **$P < 0.01$ by non-parametric test). A summarized table showing the median values of the relative abundance of phylum (%) in three groups; (B) Heatmap of the relative abundance of MTP at genus level in three groups (color key indicates taxon % abundance). The Shannon index shows the species diversity and evenness for each sample. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

showed significant differences between groups. The relative abundance of representative microbial taxa at the genus level for the three groups was visualized as a heatmap (Fig. 1B). Bacterial AGE showed very low relative abundance (%) of the causative bacteria in the gut microbiome (average abundance, the genus *Salmonella*, 0.0049%; *Campylobacter,* 1.4525%; and *Clostridium*, 2.2874%).

However, beta diversity, representing the similarity in MTP between three groups, showed significant difference (beta set significances, $P = 0.015$ by pairwise PERMANOVA with 999 permutations). In particular, the MTP differed significantly between bacterial and viral AGE ($P = 0.009$) and between bacterial AGE and convalescence ($P = 0.010$), but not between viral AGE and convalescence ($P = 0.620$).

3.3. Diversity in gut microbiome according to pathogens in pediatric AGE

The MTP of the gut microbiome in three groups of AGE, norovirus (n = 11), *Campylobacter* (n = 7) and *Salmonella* (n = 5) was compared.

The MTP in the norovirus AGE, *Campylobacter* AGE, and *Salmonella* AGE are shown in Fig. 2A. Actinobacteria was significantly more abundant in norovirus AGE (median, 10.5%, 0.7%, and 0.1%, $P = 0.011$); Proteobacteria was significantly more abundant in *Salmonella* AGE (7.1%, 13.1%, and 55.1%, $P = 0.012$), whereas Bacteroidetes was non-significantly more abundant in *Campylobacter* AGE (9.2%, 51.1%, and 2.9%, $P = 0.067$). The alpha diversity of the ACE index in norovirus AGE, *Campylobacter* AGE, and *Salmonella* AGE (median, 280, 210, and 148, $P = 0.329$) and the Shannon index (median, 2.273, 2.614, and 1.352, respectively; $P = 0.057$) showed no statistical differences (Fig. 2B), although alpha diversity was lowest in *Salmonella* AGE. Beta

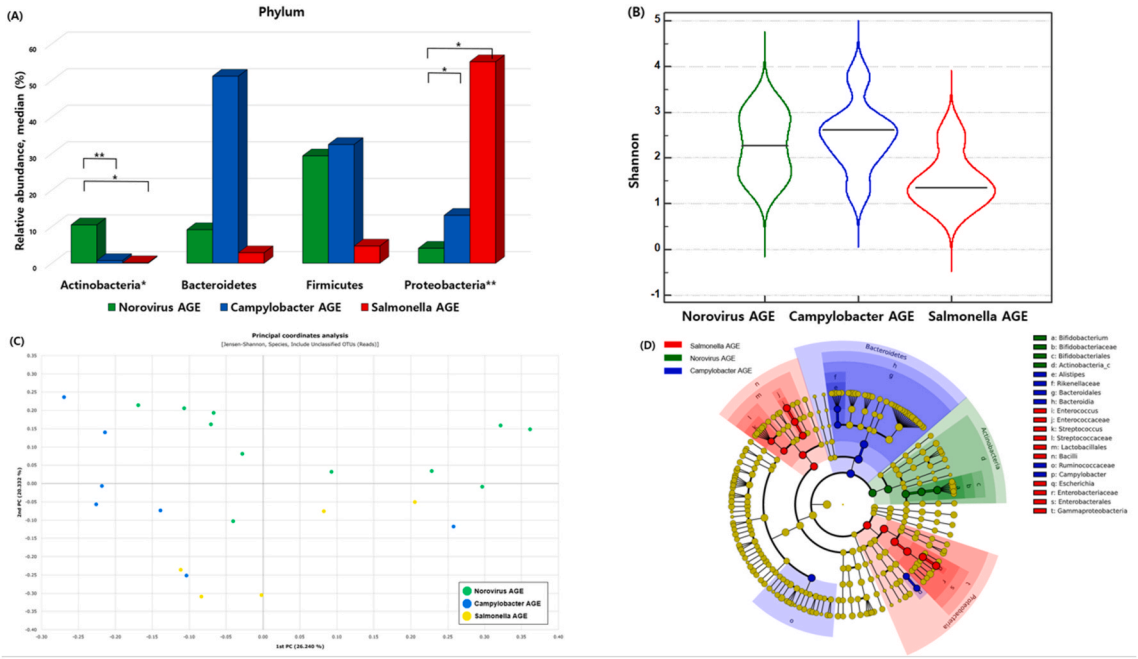

Fig. 2. Comparison of gut microbiome taxonomic composition (MTP) in pediatric patients with noroviral AGE (n = 11), *Campylobacter* AGE (n = 7), and *Salmonella* AGE (n = 5). (A) Relative abundance, Median (%, y-axis) of gut MTP at phylum level in three groups (x-axis) (post-hoc analysis * $P < 0.05$ and ** $P < 0.01$ by non-parametric test); (B) Alpha diversity indices (y-axis) of gut MTP in three groups (x-axis) (all, $p > 0.05$ by nonparametric test); (C) Principal coordinates analysis of gut MTP at species level ($P = 0.007$, by pairwise PERMNOVA with 999 permutations); (D) Cladogram based on LEfSe analysis shows the phylogenetic distribution of the taxa abundant (LEfSe size effect ≥4) in three groups.

diversity in three groups, showed significant differences at the species level ($P = 0.007$ by pairwise PERMANOVA with 999 permutations). In particular, the MTPs of norovirus and *Campylobacter* AGE ($P = 0.015$), norovirus and *Salmonella* AGE ($P = 0.003$), and *Campylobacter* and *Salmonella* AGE ($P = 0.044$) differed significantly. The PCoA plot presenting taxonomic differences at the species level in three groups is shown in Fig. 2C.

The LEfSe analysis-based cladogram displayed the phylogenetic distribution of significant taxonomic biomarkers (LEfSe≥4) in three groups (Fig. 2D). In norovirus AGE, Actinobacteria, including *Bifidobacterium* were most abundant. In *Campylobacter* AGE, Bacteroidia including *Alistipes*, Ruminococcaceae, and *Campylobacter* were most abundant. In *Salmonella* AGE, Proteobacteria including *Escherichia,* Bacilli including *Enterococcus* and *Streptococcus* were most abundant.

3.4. Changes of selected gut taxa between the acute phase of AGE and convalescence

We investigated changes in important gut bacterial taxa in 13 patients (seven with norovirus AGE and six with bacterial AGE), with paired AGE and convalescent specimens. Three of six patients with bacterial AGE had *Campylobacter* AGE and the other three had *Salmonella* AGE. Eight known important gut taxa were selected as follows: the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, and families Christensenellaceae, Lachnospiraceae, Ruminococcaceae and Enterobacteriaceae. Among them, the median abundance (%) was significantly higher during convalescence in Firmicutes (27.2% vs. 49.1%, $P = 0.003$) and families Christensenellaceae (0.000% vs. 0.003%, $P = 0.015$) and Lachnospiraceae (6.9% vs. 15.19%, $P = 0.033$) (Fig. 3). In contrast, the median abundance (%) of Proteobacteria decreased significantly during convalescence (7.5% vs. 1.6%, $P = 0.008$), and Enterobacteriaceae decreased non-significantly (3% vs. 0.9%, $P = 0.110$).

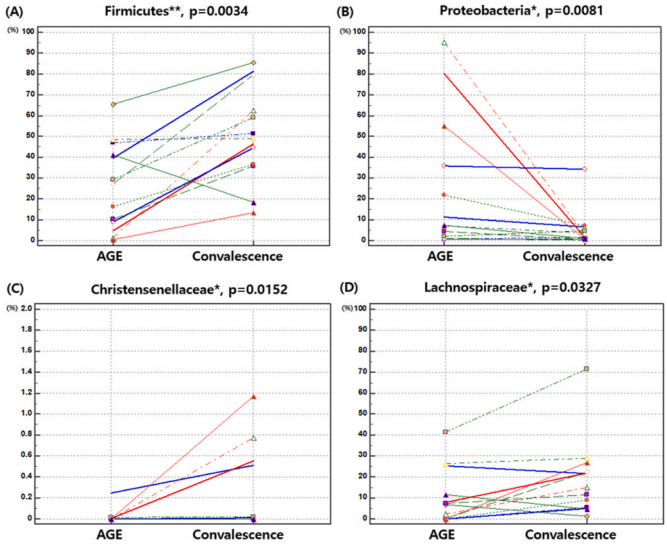

Fig. 3. Changes of relative abundance (%, y-axis) of important gut taxa in individual AGE with convalescence specimens (x-axis). Dot and line diagrams show noroviral AGE as green color (n = 7), *Campylobacter* AGE as blue color (n = 3), and *Salmonella* AGE as red color (n = 3). *$P < 0.05$ and **$P < 0.01$ by Wilcoxon test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

First, we found that the alpha diversity (species richness, and diversity) of the gut microbiome of bacterial and viral AGE was similar in the convalescence after 1 month, with no statistical difference. According to previous reports, the alpha diversity of the gut microbial community decreased in the early phase of patients with infectious AGE compared with healthy individuals [20–22]. Reduced intestinal transit

time during diarrhea promotes the reduction of obligate anaerobic gut commensals associated with short-chain fatty acids caused by the temporarily oxygenated gut environment, [22,23] and taxonomic changes in the gut microbiota occur rapidly with an increase in fast-growing facultative anaerobes [22].

In this study, the causative agents detected were norovirus, *Campylobacter*, and *Salmonella* in the order; they are representative and common causative pathogens of pediatric gastroenteritis in Korea [10–12]. The relative abundance of causative bacterial pathogens in the gut microbiome was found to be relatively low in this study, and these results are similar to those of previous studies [14,18]. However, there was a statistically significant difference in the beta diversity (taxonomic similarity) of the gut microbiota between the diarrhea causative agent groups, which is consistent with previous studies [14–18,22].

Here, when comparing the norovirus, *Campylobacter*, and *Salmonella* AGE, Actinobacteria and lower taxa, including *Bifidobacterium* were increased in norovirus AGE, and Proteobacteria and its lower taxa *Escherichia* and *Streptococcus* showed a significant increase in *Salmonella* AGE. The gut MTP of *Campylobacter* AGE was different from that of *Salmonella* AGE, as Bacteroidia and Ruminococcaceae were abundant, which differ from findings of previous reports and need to be verified in more cases [15–18].

The et al. [22] reported that different diarrheal etiologies may have triggered changes in the gut microbiome, and that bacteria-induced diarrhea is associated with an increase in fast-growing facultative anaerobes, including Proteobacteria from the Enterobacteriaceae family and *Streptococcus*, whereas viral infections retain a higher abundance of *Bifidobacterium* [22]. During an episode of diarrhea, the nature of the gut microbiome may change, with certain bacterial species increasing or decreasing depending on the cause of the diarrhea, but the changes have not yet well characterized [22]. The results may add to this knowledge.

The clinical features did not differ significantly, between bacterial and viral AGE; however, dehydration due to vomiting was the main reason for hospitalization in patients with viral AGE, and fever and diarrhea were the main reason for hospitalization in patients with bacterial AGE. Studies have reported a correlation between clinical features and the gut microbiome [15,16]. Dysbiosis in the gut microbiota and an increase in Proteobacteria is associated with inflammatory bowel disease [22]. In this study, an increase of Proteobacteria in the gut microbiome were observed in bacterial AGE patients with fever and diarrhea as the main clinical manifestations. Additionally, CRP and ESR were significantly higher in the bacterial AGE than in the viral AGE. Further research is needed to explore the relationship between Proteobacteria and gut inflammation in infectious AGE.

Second, compared with the acute phase of AGE, the convalescent samples showed a statistically significant increase in Firmicutes and its lower families Christensenellaceae and Lachnospiraceae, and a decrease in Proteobacteria. This is consistent with previous studies which have reported that Bacteroidetes and Firmicutes are more common in the healthy control group, and Proteobacteria are more common in the AGE group [17,18]. The MTP changes caused by infection are thought to recover to a healthy state over time, although the rate of recovery may vary between individuals [18]. In other words, the results of this study showed that a single AGE event was associated with a temporary disruption of the gut microbiota, but that it was restored to a healthy state without causing long-term disruption of the gut microbiota. However, the patients were only followed up for one month, so longer-term studies are needed. A range of risk factors is thought to be important for long-term disruption of the gut microbiota, including diet, medications, undernutrition, and lifestyle habits [19–21,24,25].

The gut microbiome of children varies during the first few months of life, depending on the circumstances of birth, including delivery and feeding, and is usually enriched in Firmicutes, which contain various Actinobacteria, Bacteroidetes, and Proteobacteria at the phylum level [1,5,19]. Actinobacteria has been reported to be more abundant in infants than in adults, [24] and with increasing age, around the age of 3 years, the gut microbiome converges to a typical adult-like pattern that has greater diversity of abundance and richness than that in young children, characterized by a predominance of Firmicutes and Bacteroidetes.[1,2,5,24.] In this study, the age of patients with viral AGE was significantly lower than that of patients with bacterial AGE, which is consistent with the high incidence of norovirus infections in children younger than 24 months in Korea [10–12]. In this study, Actinobacteria was increased in norovirus AGE, but the analysis was performed in a young age group (younger than 3 years of age), which may have biased the interpretation.

Firmicutes and Bacteroidetes are involved in producing short-chain fatty acids, which play a role in maintaining a healthy gut [26]. Some studies have suggested that the imbalance of Firmicutes to Bacteroidetes may be associated with obesity and metabolic disorders, e.g., type 2 diabetes [26,27]. An imbalanced gut microbiota with increased Proteobacteria might be associated with gut-related diseases [26]. Malnourished children have low levels of Bacteroides, high levels of Proteobacteria, and low microbial diversity [14,28,29]. Many species from the Bacteroidetes, Eubacteriaceae, Lachnospiraceae, and Ruminococcaceae families, which make up a healthy microbiome, are reduced and some potentially pathogenic species, such as *Escherichia*, *Enterococcus*, and *Staphylococcus* are enriched, increasing the risk of infection [14,16,28–30]. Therefore, a strategy for preventing gut disease may include probiotic therapy to maintain healthy gut microbiota and reduce the levels of Proteobacteria and *E. coli* in the gut during acute infections, while increasing microbes, including Firmicutes and Bacteroidetes, which are most important for restoring gut health [14,18,21,28].

The limitations of this study are as follows: first, a small number of the data might not be sufficient to be representative of infectious acute gastroenteritis. In agreement with a previous study [31] as the study period overlapped with the COVID-19 pandemic, social distancing and public hygiene were strictly enforced which lowered the incidence of AGE and the number of enrolled subjects was small. Second, we did not record the history of probiotic use, which can influence the commensal microbiome. Third, there was no healthy controls group, and although convalescent samples may be typical of the host, they may not be representative of the gut microbiome composition in the pediatric age group.

In conclusion, we found that viral and bacterial AGE affect the diversity and dominant taxa of the gut microbiota in pediatric patients. Healthy gut bacteria increased during the convalescent period 1 month after infection, indicating that a single AGE event can cause transient disruption of the gut microbiome but the gut returns to a healthy state without AGE causing long-term dysbiosis.

Funding

This research was supported by a grant from the Translational R&D Project of the Institute for Biomedical Convergence, Incheon St. Mary's Hospital, Catholic University of Korea.

Authorship

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication.

Indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Chang). The name of each author must appear at least once in each of the three categories below.

Category 1

Conception and design of study (typed): Sang Yong Kim, Seungok

Lee, Myungshin Kim; acquisition of data (typed): You Ie Kim, Sang Yong Kim, Seungok Lee; analysis and/or interpretation of data (typed): You Ie Kim, Seungok Lee, Myungshin Kim.

Category 2

Drafting the manuscript (typed): You Ie Kim, Seungok Lee; revising the manuscript critically for important intellectual content (typed): Sang Yong Kim, Myungshin Kim, Woo Jin Kim.

Category 3

Authorship

Seungok Lee ORCID : 0000-0003-4538-8427.

Declaration of competing interest

The authors report no conflict of interest.

References

[1] Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A 2011;108(Suppl 1):4578–85.
[2] Ihekweazu FD, Versalovic J. Development of the pediatric gut microbiome: Impact on health and disease. Am J Med Sci 2018;356:413–23.
[3] Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. Role of the microbiome in human development. Gut 2019;68:1108–14.
[4] Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 2015;17:690–703.
[5] Odamaki T, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao JZ, et al. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. BMC Microbiol 2016;16:90.
[6] Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev 2017;81:e00036-17.
[7] Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, et al. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. JAMA Pediatr 2017;171:647–54.
[8] GBD 2016 Diarrhoeal Disease Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 2018;18:1211–28.
[9] Hartman S, Brown E, Loomis E, Russell HA. Gastroenteritis in children. Am Fam Physician 2019;99:159–65.
[10] Ryoo E. Causes of acute gastroenteritis in Korean children between 2004 and 2019. Clin Exp Pediatr 2021;64:260–8.
[11] Cho SR, Chae SJ, Jung S, Choi W, Han MG, Yoo CK, et al. Trends in acute viral gastroenteritis among children aged ≤5 years through the national surveillance system in South Korea, 2013-2019. J Med Virol 2021;93:4875–82.
[12] Kim A, Chang JY, Shin S, Yi H, Moon JS, Ko JS, et al. Epidemiology and factors related to clinical severity of acute gastroenteritis in hospitalized children after the introduction of rotavirus vaccination. J Korean Med Sci 2017;32:465–74.
[13] Lin PC, Yang YSH, Lin SC, Lu MC, Tsai YT, Lu SC, et al. Clinical significance and intestinal microbiota composition in immunocompromised children with norovirus gastroenteritis. PLoS One 2022;17:e0266876.
[14] Taco-Masias AA, Fernandez-Aristi AR, Cornejo-Tapia A, Aguilar-Luis MA, Del Valle LJ, Silva-Caso W, et al. Gut microbiota in hospitalized children with acute infective gastroenteritis caused by virus or bacteria in a regional Peruvian hospital. PeerJ 2020;8:e9964.
[15] Mathew S, Smatti MK, Al Ansari K, Nasrallah GK, Al Thani AA, Yassine HM. Mixed viral-bacterial infections and their effects on gut microbiota and clinical illnesses in children. Sci Rep 2019;9:865.
[16] Gallardo P, Izquierdo M, Vidal RM, Chamorro-Veloso N, Rosselló-Móra R, O'Ryan M, et al. Distinctive gut microbiota is associated with diarrheagenic *Escherichia coli* infections in Chilean children. Front Cell Infect Microbiol 2017;7:424.
[17] Chen SY, Tsai CN, Lee YS, Lin CY, Huang KY, Chao HC, et al. Intestinal microbiome in children with severe and complicated acute viral gastroenteritis. Sci Rep 2017;7:46130.
[18] Singh P, Teal TK, Marsh TL, Tiedje JM, Mosci R, Jernigan K, et al. Intestinal microbial communities associated with acute enteric infections and disease recovery. Microbiome 2015;3:45.
[19] Ronan V, Yeasin R, Claud EC. Childhood dvelopment and the microbiome-the intestinal microbiota in maintenance of health and development of disease during childhood development. Gastroenterology 2021;160:495–506.
[20] Dixit K, Chaudhari D, Dhotre D, Shouche Y, Saroj S. Restoration of dysbiotic human gut microbiome for homeostasis. Life Sci 2021;278:119622.
[21] Gaufin T, Tobin NH, Aldrovandi GM. The importance of the microbiome in pediatrics and pediatric infectious diseases. Curr Opin Pediatr 2018;30:117–24.
[22] Chung The H, Le SH. Dynamic of the human gut microbiome under infectious diarrhea. Curr Opin Microbiol 2022;66:79–85.
[23] Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. Science 2016;352:560–4.
[24] Haran JP, McCormick BA. Aging, frailty, and the microbiome-How dysbiosis influences human aging and disease. Gastroenterology 2021;160:507–23.
[25] Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. Cell Mol Life Sci 2017;74:2959–77.
[26] Pascale A, Marchesi N, Marelli C, Coppola A, Luzi L, Govoni S, et al. Microbiota and metabolic diseases. Endocrine 2018;61:357–71.
[27] Komaroff AL. The microbiome and risk for obesity and diabetes. JAMA 2017;317:355–6.
[28] Pekmez CT, Dragsted LO, Brahe LK. Gut microbiota alterations and dietary modulation in childhood malnutrition - the role of short chain fatty acids. Clin Nutr 2019;38:615–30.
[29] Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, et al. Gut microbiota of healthy and malnourished children in Bangladesh. Front Microbiol 2011;2:228.
[30] Million M, Tidjani Alou M, Khelaifia S, Bachar D, Lagier JC, Dione N, et al. Increased gut redox and depletion of anaerobic and methanogenic prokaryotes in severe acute malnutrition. Sci Rep 2016;6:26051.
[31] Chang ES, Yeh PJ, Chen CC, Lai MW, Chao HC, Wu HK. Effect of the coronavirus disease 2019 pandemic on pediatric emergency department visits for acute gastroenteritis evaluated using a validated clinical severity score. Pediatr Neonatol 2022;64:381–7.