

## The Comparison of Clinical Characteristics in Three Types of Viral Acute Diarrhea in Infants and Toddlers and the Effect of *Lactobacillus acidophilus* on Rotaviral Diarrhea

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The most common causes of acute viral diarrhea in infants and toddlers are rotavirus, astrovirus and norovirus. The purpose of this study was to evaluate epidemiological data of pathogens obtained from stool exams and compare them with the clinical course in pediatric patients with symptoms of viral acute diarrhea and to investigate the clinical efficacy of *Lactobacillus acidophilus* (*L. acidophilus*) for acute diarrhea caused by rotavirus. Clinical data for three types of viral acute diarrhea were compared with the viral detection results by enzyme-linked immunosorbent assay. Viruses were detected in 65.8% of subjects, with rotavirus being the most commonly reported in 63.3% of subjects. To examine the characteristics of each virus, a clinical epidemiological study was performed for 482 cases. Noroviral infection symptoms included vomiting and diarrhea in patients of all age groups. Dehydration in noroviral acute diarrheal patients was less common than in rotaviral acute diarrheal patients. The clinical efficacy of orally administered *L. acidophilus* in the treatment of acute viral diarrhea in infants and toddlers was also evaluated. *L. acidophilus* was a probiotic adjuvant in viral acute diarrhea in infants and toddlers.

**Key Words:** Viral diarrhea, Rotavirus, Astrovirus, Norovirus, *Lactobacillus acidophilus*

No potential conflict of interest relevant to this article was reported.

## INTRODUCTION

Diarrhea is the second most common illness in infants and toddlers, second only to respiratory infections, and is classified as acute or chronic based on its duration, i.e., according to whether diarrhea lasts less than two weeks or not. The causes of diarrhea are divided into infectious diarrhea due to bacteria and viruses and non-infectious diarrhea, associated with factors such as the use of antibiotics, malnutrition, and food allergy. Most cases of diarrheal diseases in infants and toddlers are due to viral infection.

Viral gastroenteritis develops rapidly and causes acute diarrhea with or without symptoms such as vomiting, fever, chronic diarrhea, and abdominal pain. The most common cause of acute gastroenteritis in infants and toddlers is rotavirus; norovirus and astrovirus are also among the common causes. In addition, enteric adenoviruses, enteroviruses, and coronaviruses are also known to cause gastroenteritis in various ways depending on the age, region, and season.

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Rotavirus is the most important causative pathogen of infantile diarrhea in both advanced and developing countries. Rotavirus enters the body through the fecal-oral route and infects the epithelial cells in the upper small intestine. During the two to four days incubation period, rotavirus changes the function of the small intestine epithelium and thereby causes lesions mainly in digestive organs, resulting in vomiting, fever and mild diarrhea. If the rotavirus infection is asymptomatic or not properly treated during the early phase of infection, it may lead to life-threatening severe diarrhea and dehydration through absorption failure due to cellular damage, NSP4 enterotoxin, and stimulation of the enteric nervous system. Patients may also experience electrolyte disturbance and metabolic acidosis. Recently, viral infection has also been reported in tissues other than the gastrointestinal tract. Vandenplas *et al.* reported that rotavirus antigens were detected in the stomach, intestines, liver, kidneys, urinary bladder, thymus, and pancreas of infected mice (1). In addition, Oh *et al.* reported that rotavirus was also present in the blood and intestines of individuals with rotavirus infection (2). These studies have shown that rotavirus not only induces diarrhea in the digestive organs, but also is capable of replication and excretion in other organs and may cause other diseases.

Norovirus has been reported to be the most important cause of food-borne illness in institutional food service settings. Approximately 47% of infants infected with norovirus were children aged 6 to 18 months, and the major cause of gastroenteritis in infants under the age of 5 was norovirus infection. Norovirus causes symptoms such as vomiting and diarrhea in all age groups including infants and toddlers. As the proportion of institutional food services has steadily increased, the damage of mass food poisoning outbreaks caused by norovirus has also increased. Norovirus has a short incubation period of 12~48 hours and the morbidity is as short as 1~3 days, but nausea and vomiting are prominent and symptoms are similar to those of bacterial food poisoning. Norovirus infection in infants resembles rotavirus gastroenteritis and shows a slight progress, and the rate of spontaneous recovery is high. However, since the person-to-person transmission rate is high, secondary infection occurs easily and may cause a mass outbreak.

Astrovirus infection is found throughout the year but occurs frequently in winter. Community-based research showed that astrovirus infections account for 5~17% of infants with diarrhea and 2~16% of infants admitted to the hospital due to diarrhea (3). Astrovirus infection usually involves one or more symptoms such as diarrhea, vomiting, fever, abdominal pain, and headache. Symptoms occur within 2~3 days after infection and last for 2~3 days; dehydration is observed only in exceptional cases.

The rotavirus vaccines approved for use are a live attenuated monovalent vaccine Rotarix® and a live pentavalent, human-bovine reassortant vaccine Rotataq®. It is difficult to defend against the wide range of Rotavirus serotypes using vaccines, and the high costs associated with a vaccine program also cause many difficulties (4).

*Lactobacillus* spp. are a nonmotile, gram-positive, asporogenous bacillus that produces organic acids by decomposing glycogen; it is among the representative microorganisms that have been used for fermentation. *Lactobacillus* strains are resistant to acid and have intestinal regulation, anti-cancer, blood cholesterol lowering, and vitamin B group synthetic effects. It can adhere to the human gastrointestinal tract and can adapt to and survive in the intestinal environment, thus playing an important role in maintaining intestinal microbial homeostasis. Using the characteristics of these *Lactobacillus* strains, studies have been carried out *in vitro* and *in vivo* on the inhibition of adhesion and replication of various viruses, including rotavirus, or the prevention of infection. Lin *et al.* reported that *L. rhamnosus* GG increased the rate of clearance of rotavirus from the body, and the inhibitory effect of *L. paracasei* against rotavirus was also reported (5). *L. rhamnosus* GG is also known to promote the recovery from acute diarrhea and is effective in the treatment of rotavirus gastroenteritis. *L. delbrckii* subsp. *bulgaricus* has also been reported as a treatment for rotavirus gastroenteritis. In addition, *L. reuteri* has been reported to effectively treat acute diarrhea and rotavirus gastroenteritis (6~8).

The aim of this study was to compare the clinical characteristics of three types of viral infections in pediatric patients admitted to the hospital for acute gastroenteritis and to investigate the clinical efficacy of *L. acidophilus* for acute diarrhea caused by rotavirus.

## MATERIALS AND METHODS

### Study design and population

Human studies conducted in this study was approved by the Institutional Review Board at Duksung Women's University (IRB approval number; 2018-1211-02-01). The purpose of this study was to compare the clinical characteristics of three types of viral infections in pediatric patients admitted to the hospital for acute gastroenteritis. Among children admitted to the university hospital from June 2014 to May 2016, we collected stool specimens from 734 patients with diarrhea and performed a stool test. As a result, viruses were detected in fecal samples from 483 patients who were then included in this study, and their clinical symptoms were investigated.

Among children aged 3 months to less than 5 years admitted to a university hospital from August 2016 to October 2016, rotavirus was detected in stool specimens from 30 pediatric patients. After obtaining informed consent for participation, these patients were included in the study to investigate the effect of *L. acidophilus* (ATCC 4357) on acute diarrhea due to rotavirus in infants and young children. A 10-fold dilution of 100 mg of fecal sample was produced by phosphate-buffered saline (PBS, pH 7.4, Sigma-Aldrich, St. Louis, MO, USA); the sample was centrifuged at 3,000 rpm for 20 minutes at 4°C, and the supernatant was taken and used.

The enzyme-linked immunosorbent assay (ELISA) was used to detect the antigens of rotavirus, norovirus, and astrovirus. The detection of the rotaviral antigens was carried out by using the rotavirus detection kit (BIOINCELL, Houston, TX, USA). And the detection of the noroviral antigens was carried out by using the RIDASCREEN norovirus ELISA kit (R-Biopharm AG, Darmstadt, Germany). Also the detection of the astroviral antigens was carried out by using the IDEIATM kit (DAKO, Glostrup, Denmark), and measurements were carried out according to each manufacturer's recommendations (9).

### Evaluation of clinical efficacy of *L. acidophilus* treatment

Pediatric patients who had acute diarrhea with a duration of seven days or less and who had diarrhea once or more within 24 hours before admission were included in the study. The participants were randomly divided into two groups. The *L. acidophilus* group (n=16) was given  $0.5 \times 10^8$  CFU of *L. acidophilus* orally twice a day and non-fat dry milk power was given in the placebo group (n=14). *L. acidophilus* (ATCC 4357) and placebo were manufactured by Hanmi Central Research Institute (Giheung-si, Korea). The efficacy of treatment was evaluated by examining the number of diarrhea episodes, fever and vomiting at the same time periods every day after admission. The duration of diarrhea from admission to the last diarrhea were recorded.

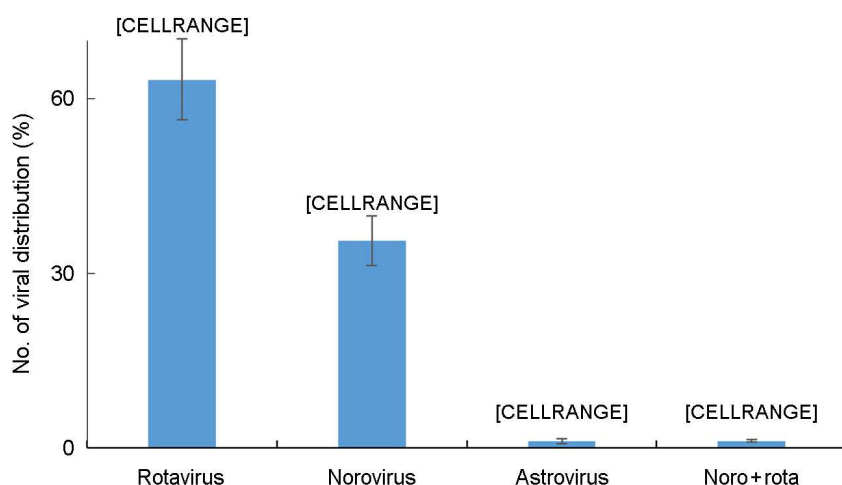
Statistical analysis was performed using SPSS version 19.0; *t*-test and chi-square test were performed to determine statistical significance. If the *p*-value was less than 0.05, the difference between two groups was considered statistically significant.

## RESULTS AND DISCUSSION

### Comparison of the clinical characteristics of three types of viral diarrhea

According to the viral gastroenteritis monitoring system, group A rotavirus gastroenteritis begins to increase in December every year and is isolated most frequently in February and March. Norovirus starts to increase in November, occurring most frequently in December and January but is found all year round. Japanese study reported that rotavirus accounted for the highest proportion of cases (43.6%), followed by norovirus (29.9%), adenovirus (7.6%) and astrovirus (1.6%) (10).

The current study detected viruses in 483 samples after examination of fecal samples collected from 734 infants and toddlers admitted to a university hospital from June 2014 to May 2016. Their main symptom was viral acute diarrhea, and we investigated



**Figure 1.** Distribution of rotavirus, norovirus and astrovirus from infants and toddlers with acute diarrhea.

**Table 1.** Sex ratio of patients with diarrhea

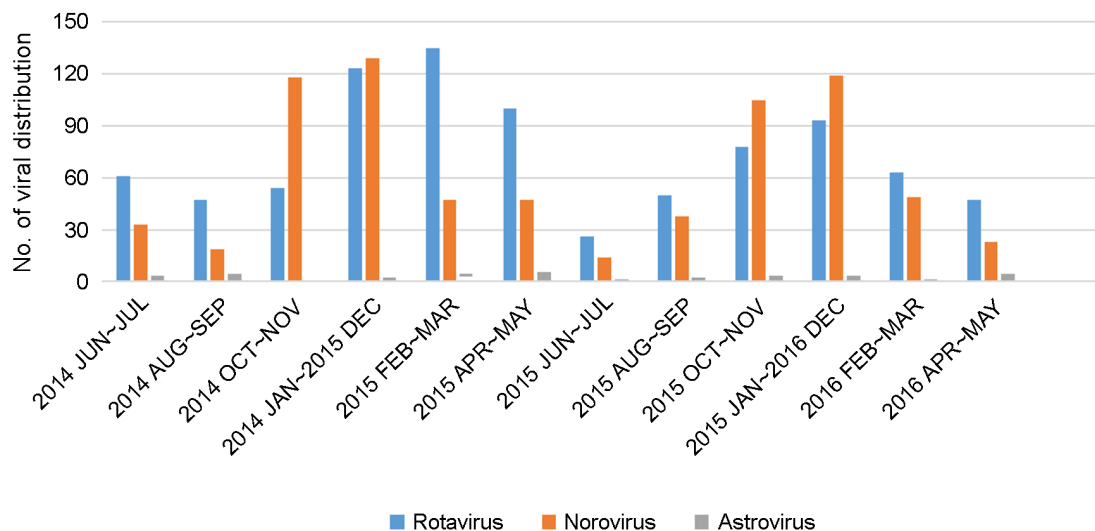
	Rotavirus	Norovirus	Astrovirus	<i>p</i> value
No.	305	171	6	NS
Male (%)	53	68	66.7	NS
Female (%)	47	32	33.3	NS
Mean age (Mo.)	16.1±16.54	15.4±9.25	11.0±0.15	NS

the clinical symptoms of the subjects. Rotavirus was common cause (63.3%), followed by norovirus (35.5%) and astrovirus (1.2%) (Fig. 1).

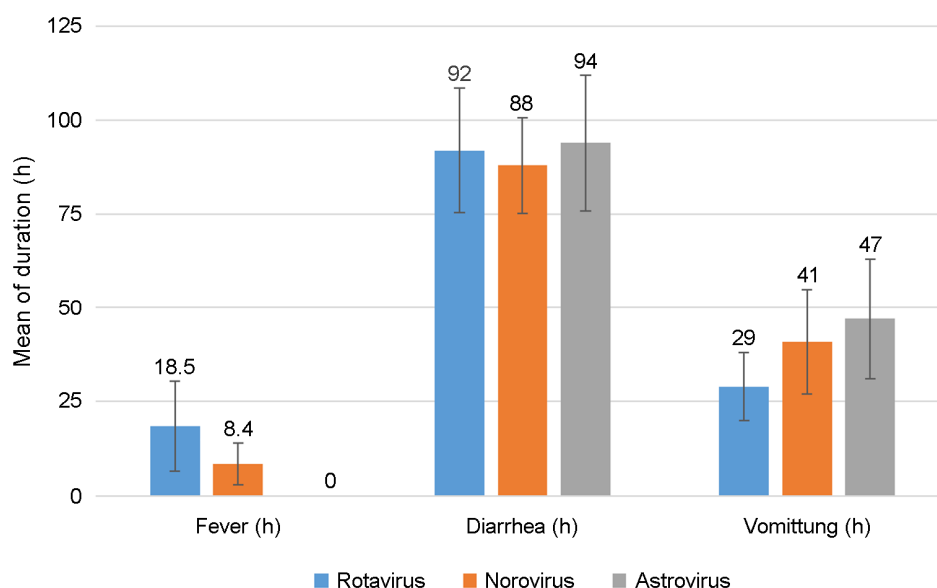
Among patients with rotavirus infection, 53% were boys and 47% were girls. Among those with norovirus infection, 68% were boys and 32% were girls, while among those with astrovirus infection, 66.7% were boys and 33.3% were girls. Rotavirus infection mainly has been reported that it occurs mainly in children aged 0 to 12 months in developing countries and in infants aged 12 to 24 months in advanced countries (11). In the present study, the mean age of all the pediatric patients was 16.1 months, and the mean age of the patients with norovirus and astrovirus infection were 15.4 months, and 11 months, respectively. Although there was no significant difference, the mean age of patients was somewhat lower in those with astrovirus infection than other viruses (Table 1).

Rotavirus gastroenteritis is known to occur mainly in late autumn and early winter. According to the published studies in Korea, it begins to occur in November and December, and a high rotavirus positivity rate is observed even in May. In this study, the incidence of rotavirus gastroenteritis increased from November and reached a peak in March and even in May. These results are similar to previous studies. Norovirus was detected until May, and although the number of pediatric patients with astrovirus gastroenteritis was small, it was highest in May (Fig. 2). Astrovirus gastroenteritis is also caused by fecal-oral transmission and is associated with shorter duration and less serious diarrhea than other intestinal viruses. In temperate climate regions, it is known to be prevalent in winter, but no characteristic seasonal distribution was observed in this study (Fig. 2).

The Fig. 3 summarizes the duration of fever in pediatric patients with gastroenteritis. The duration of fever was 0.77 days for



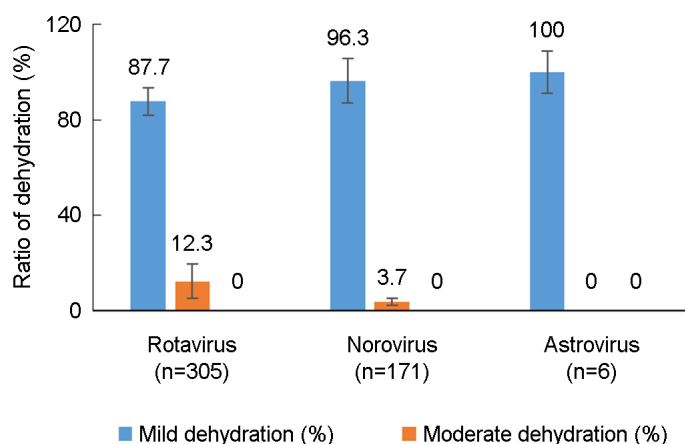
**Figure 2.** Seasonal distribution of rotavirus, norovirus and astrovirus from infants and toddlers with acute diarrhea.



**Figure 3.** Mean duration of fever, diarrhea, vomiting of rotavirus, norovirus and astrovirus from infants and toddlers with acute diarrhea.

rotavirus and 0.35 days for norovirus; no patients with astrovirus infections had fever (Fig. 3). In the case of rotavirus infection, the duration of vomiting was 1.2 days, and that of diarrhea was 3.82 days. For norovirus infection, the duration of vomiting was 1.71 days and that of diarrhea was 3.66 days. For astrovirus infection, the duration of vomiting was 2 days and that of diarrhea was 4 days (Fig. 3). Although rotavirus infection was reported to cause more severe fever and diarrhea in both frequency and duration but less serious vomiting compared to norovirus infection, the symptoms of two types of viral infections were similar in this study (12).

Norovirus infections are caused by ingestion of virus particles or by airborne transmission, and nausea, vomiting and diarrhea



**Figure 4.** Distribution of dehydration of rotavirus, norovirus and astrovirus from infants and toddlers with acute diarrhea.

occur after a short incubation period of 12~48 hour. While rotavirus and astrovirus mainly cause infantile gastroenteritis in children under 5 years of age, norovirus infections occur in various age groups and are a causative agent of mass outbreaks related to institutional food services such as school meal services. Most patients show mild symptoms, but the secondary attack rate is high, and easily transmitted, there is a very strong possibility of mass outbreaks. Typical symptoms of acute gastroenteritis caused by norovirus are sudden vomiting and diarrhea following a 24~48 hours incubation period; fever may also occur. The symptoms of noroviral gastroenteritis are similar to those of rotaviral gastroenteritis. However, a study of inpatients in Korea reported that more severe vomiting was observed in norovirus infections, while diarrhea was more severe and persistent in rotavirus infections (13).

A comparison of the degrees of dehydration in the cases of viral gastroenteritis according to the causative virus showed that rotavirus caused mild dehydration in 87.7% of patients, moderate dehydration in 12.3%, and serious dehydration in no patients. As for patients infected with norovirus, mild dehydration was observed in 96.3% of patients, moderate dehydration in 3.7%, and severe dehydration in none. In the cases of astrovirus infection, all pediatric patients had mild dehydration (Fig. 4). To prevent dehydration, oral rehydration solution (ORS), which basically replaces the lost fluids, is administered. The patient with vomiting or serious dehydration is treated by intravenous replacement therapy. ORS may decrease mortality from dehydration and acidosis, but it is difficult to reduce the duration of acute diarrhea using ORS. The treatment of rotavirus gastroenteritis is also focused on the treatment of dehydration due to diarrhea and vomiting, and supportive care such as ORS or nutrition supply is important.

Asymptomatic infection occurs in 40% of infants and toddlers infected with rotavirus, and the rate of asymptomatic infection is higher in neonates (14). Immunity against rotavirus infection is divided into maternal immunity and protective immunity after natural infection. Protective immunity after natural infection has cross-immunity other serotypes as well as the serotypes that caused the primary infection. There is no special treatment for viral infections, and mass outbreaks need to be prevented through rapid screening and active infection management. The World Health Organization (WHO) has reported that treatment effects were observed in 90% of pediatric patients treated only by the correction of electrolyte imbalance by ORS due to dehydration and food intake; no other specific medications or antibiotics are recommended (15). The use of inappropriate antibiotics and antidiarrhea may cause chronicity of symptoms or serious side effects rather than improvement of diarrhea.

### Clinical efficacy of *L. acidophilus* for acute diarrhea caused by rotavirus

To effectively treat acute diarrhea, it is necessary to shorten the duration of diarrhea, improve the nutritional status of the patient,

**Table 2.** Frequency and duration of diarrhea and vomiting with *L. acidophilus* in infant and toddler patients with acute rotaviral diarrhea

	Placebo (n=16)	<i>L. acidophilus</i> (n=14)	<i>p</i> value
Fever (day)	4.32±1.94	3.66±1.14	0.118
Diarrhea (day)	5.56±1.13	4.38±1.24	0.071
Diarrhea 1 day (No.)	6.41±2.9	4.09±2.0	0.046
Diarrhea 3 day (No.)	4.15±2.5	1.94±1.1	0.021
Vomiting 2 day (No.)	6.40±4.2	2.38±1.1	0.031
Vomiting 3 day (No.)	4.26±3.8	0.88±1.1	0.046

and strengthen the intestinal mucous membrane protective layer. Probiotics and metabolites of lactic acid bacteria are known to play an important role in the colonization of intestinal microorganisms and in establishing and maintaining their balance in adults as well as infants and toddlers (16). Their major benefits include improving the balance of intestinal normal flora of the host, enhancing immunity, competitively inhibiting intestinal epithelial cell adhesion of pathogenic bacteria, and secreting antimicrobial substances. LAB and its metabolites are known to stimulate nonspecific immunity or contribute to the activation of immune responses, and they are known to compete with infectious viruses or microorganisms for adherence to epithelial cells in gastroenteritis, thereby reducing the pathogenicity and shortening the duration of diarrhea (17).

To evaluate the clinical efficacy of LAB, *L. acidophilus*, in acute diarrhea caused by rotavirus in infants and toddlers, 30 pediatric patients aged 3 months to less than 5 years who were admitted to a university hospital from August to October 2016 and positive for rotavirus were included in the study after obtaining the informed consent to participate. The subjects, who were the rotavirus antigen-positive group, were divided into the *L. acidophilus* group (n=16) and the placebo group (n=14). The *L. acidophilus* group and the placebo group were matched for age, and the mean age of the subjects was 19.5 ± 6.8 and 19.4 ± 8.5 months, respectively.

The mean duration of diarrhea was 4.32 ± 1.45 days in the *L. acidophilus* group and 5.56 ± 1.3 days in the placebo group, but the difference was not significant ( $p = 0.071$ ) (Table 2). On the first day of treatment, the mean number of diarrhea episodes was 4.09 ± 2.0 in the *L. acidophilus* group and 6.41 ± 2.9 in the placebo group, and the difference between the two groups was statistically significant ( $p = 0.046$ ). On the third day of treatment, the number of diarrhea episodes was 1.9 ± 1.1 in the *L. acidophilus* group and 4.15 ± 2.5 in the placebo group ( $p = 0.021$ ), and the difference between the two groups was statistically significant (Table 2). On the second day of treatment, 16% and 40% of patients in the *L. acidophilus* group and in the placebo group had vomiting, respectively ( $p = 0.031$ ). On the third day of treatment, 6.2% of patients in the *L. acidophilus* group and 26.6% in the placebo group ( $p = 0.046$ ) had vomiting (Table 2).

LAB such as *L. casei*/GG have been reported to reduce the frequency and duration of diarrhea by 30~70% in pediatric patients infected with rotavirus (18). There were found to be effective in the prevention and treatment of rotavirus infection. Gutierrez-Castrellon *et al.* found that when *L. reuteri* was administered for two days to pediatric patients infected with rotavirus, the proportion of patients with persistent diarrhea decreased to 48%, compared to 80% in the placebo group (19). Han *et al.* reported that the level of specific IgG and IgA for the cytoplasm and cell wall of LAB were significantly higher in the group administered *Bifidobacterium longum* and *L. acidophilus* than in the control group (17). Van *et al.* reported that both the duration of diarrhea was reduced and the number of IgA secreting cells against rotavirus was significantly increased in pediatric patients to whom LAB were administered (20). Orally administered LAB pass through the intestinal lumen and activate the intestinal immune system to increase production of mucosal IgA, which inhibits the colonization of pathogenic bacteria and the invasion of serious pathogenic antigens in the case of intestinal infections.

Each species of LAB including *L. acidophilus* has different effects, which suggests that not all LAB are effective for human diseases and a mixture of LAB may not be effective. The use and dosages of LAB should be differentiated according to the cause of diarrhea symptoms. However, since the precise mechanism of LAB strains has not yet been elucidated, further research is needed.

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