Epidemiologic Changes in Over 10 Years of Community-Acquired Bacterial Enteritis in Children

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ABSTRACT

Purpose: Community-acquired bacterial enteritis (CABE) is a common problem in developed countries. It is important to understand the epidemiologic changes in bacterial pathogens for prevention and treatment. Therefore, we studied the epidemiologic changes in CABE in Korean children.

Methods: A total of 197 hospitalized pediatric patients aged <19 years that presented with dysentery symptoms and showed positive polymerase chain reaction results for bacterial species in stool samples, were enrolled in this study for 10 years (June 2010 to June 2020). We classified patients in phase I (06, 2010–06, 2015) and phase II (07, 2015–06, 2020) and analyzed their epidemiologic and clinical characteristics.

Results: The most common pathogens were Campylobacter species (42.6%) and Salmonella species were the second most common pathogens (23.9%). The abundance of pathogens decreased in the following order: Clostridium difficile (9.6%), Shigella (5.6%), and Clostridium perfringens (5.6%). Escherichia coli O157:H7 was found to be the rarest pathogen (2.0%). Campylobacter species showed an increase in the infection rate from 32.1% in phase I to 49.6% in phase II (p=0.0011). Shigella species showed a decline in the infection rate in phase I from 14.1% to 0.0% in phase II (p<0.001). C. difficile and C. perfringens showed an increase in infection rate in phase II compared to phase I, but the difference was not statistically significant.

Conclusion: The infection rate of Campylobacter species in CABE has been rising more recently, reaching almost 50%. This study may help establish policies for prevention and treatment of CABE in Korean children.

Keywords: Campylobacter; Enteritis; Community acquired infection
INTRODUCTION

Diarrhea is mainly caused by viruses, bacteria, and parasites, and the prevalence of pathogens differs according to factors such as the environment and socio-economic status [1]. Infectious diarrhea can originate from food, water, or person-to-person contact [2]. Although diarrheal diseases have been declining as a cause of death in children in developed countries, it remains a major cause of mortality and morbidity in children in resource-limited countries [1]. Bacterial pathogens are a major source of foodborne diseases in many communities [2-6]. Foodborne diseases are a major public health issue and a source of community-acquired infections (CAIs) [5]. Information dissemination and surveillance for foodborne diseases have been performed by the World Health Organization and individual countries, such as by the Center for Disease Control and Prevention (CDC) in the United States and the Korea Disease Control and Prevention Agency (KDCA) in Korea.

Rotavirus is a major causative pathogen of acute diarrhea in children worldwide; however, bacterial pathogens appear to differ according to geographical area [7]. In particular, foodborne bacterial infections are an important public health issue for community-acquired bacterial enteritis (CABE) [5]. This issue is not confined to resource-limited countries. The diversity and transmission of foodborne pathogens are associated with climate change, and foodborne diseases can easily occur during outbreaks through hotels, cruise ships, resorts, childcare centers, and restaurants [5]. According to the CDC in the United States, the incidence of bacterial enteric infection through food in 2019 increased compared to 2016–2018 [8]. The most common pathogens were Campylobacter (19.5 cases per 100,000 population), followed by Salmonella (17.1), Shiga toxin-producing Escherichia coli (STEC, 6.3), Shigella (4.8), and Yersinia (1.4) [8]. The KDCA has also investigated foodborne diseases in Korea and found that the number of occurrences of foodborne infectious diseases has been steadily increasing since 2013, according to epidemiological investigation of infectious diseases in the Korea annual report 2018.

The variety and diversity of multiple pathogens in infectious diarrhea make diagnosis problematic. Stool culture, which is used as a conventional method for finding pathogens, requires specific media, environment, and a long time. These problems lead to a change in conventional methods of detection such as culture to molecular assays to detect pathogens. The stool polymerase chain reaction (PCR) test is the gold standard for identifying organisms causing infectious diarrhea in Korea. Multiplex PCR is especially useful for the simultaneous detection of multiple organisms, such as viruses and bacteria. The FilmAarray system developed recently, is a more useful multiplex PCR test that can detect multiple pathogens faster [9].

Even in countries with high economic status, the incidence of food-borne infectious diarrhea continues to appear without decreasing. To cope with this problem, continuous epidemiologic investigations on the source of the outbreak must be conducted. These systems allow us to control and prevent outbreaks and provide physicians with information and clues for appropriate management and antibiotics. In Korea, the KDCA has established a monitoring system for the outbreak of bacterial enteritis in each region and has published epidemiological data every year. However, epidemiological investigations based on age and date are insufficient. Therefore, we aimed to identify the epidemiologic characteristics for CABE in children, in this study.
MATERIALS AND METHODS

Subjects
Patients aged 0–18 years were included in this study. A total of 321 patients were admitted to Dankook University Hospital with dysentery symptoms, of which 197 patients showed positive results for stool PCR from June 2010 to June 2020. They had no underlying diseases, coinfection with viruses, or nosocomial infections.

Methods
1. Stool samples and DNA extraction
Stool was collected from patients with dysentery symptoms within the same day or at least 2 days after hospitalization and before using medication such as antibiotics. Samples were immediately delivered to the laboratory office and stored at 2–8°C. DNA was extracted from stool samples using the QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD, USA) within 24 hours after stool collection, and stool culture was performed using standard media for Salmonella, Shigella, Escherichia coli O157:H7, and Vibrio species.

2. PCR
Multiplex PCR tests were performed using the Seeplex® Diarrhea-B1 and B2 ACE Detection kit (Seegene, Seoul, Korea). This kit can detect ten pathogen species simultaneously, including Campylobacter species, Clostridium difficile toxin B, Aeromonas species, Clostridium perfringens, E. coli O157:H7, Salmonella species, Shigella species, Verocytotoxin-producing E. coli, Vibrio species, and Yersinia enterocolitica. Multiple Master Mix (DNA polymerase, buffer with deoxynucleoside triphosphates, stabilizer, and MgCl₂) and 3 µL of nucleic acid were added to the PCR tube, and PCR was performed using a PCR machine (BioRAD, Hercules, CA, USA). The amplified products were separated according to size using a capillary electrophoresis system. We considered the PCR assay as the gold standard for the diagnosis of bacterial enteritis in this study.

3. Data analysis and statistics
The patients were classified into phases I and II. Phase I ran from June 2010 to June 2015 and Phase II ran from July 2015 to June 2020. The epidemiologic and clinical characteristics were retrospectively analyzed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Co., Armonk, NY, USA). Fisher’s exact test and analysis of variance or Mann–Whitney U-test were used to compare continuous and categorical variables between the two groups. Statistical significance was considered at \( p < 0.05 \).

4. IRB
This study was approved by the Institutional Review Board of Dankook University Hospital (IRB No. 2021-04-031-001).

RESULTS

The causative pathogens and the time of infection
The most common pathogen of CABE was Campylobacter species (84 cases, 42.6%) and Salmonella species are the second most common pathogen (47 cases, 23.9%). In the following order, C. difficile (19 cases, 9.6%), Shigella (11 cases, 5.6%), C. perfringens (11 cases, 5.6%), Aeromonas (11 cases, 5.6%), and E. coli O157:H7 was the rarest pathogen (4 cases,
The month of August was the most common time for infection (28 cases, 14.2%), and 60.9% of infections occurred between June and October (Fig. 2).

### The difference in frequency of infectious pathogens according to phase
Bacterial pathogens induced in enteritis showed differences in frequency (Fig. 3). *Campylobacter* species were the most frequent pathogens in both phase I (06, 2010–06, 2015) and phase II (06, 2016–06, 2020) (Fig. 3). In particular, the phase II period showed a significant increase in *Campylobacter* infection from 32.1% in phase I to 49.6% of the total pathogens in phase II \((p=0.011)\). *Shigella* species showed a decline in the infection rate in phase I from 14.1% to 0.0% in phase II \((p=0.001)\). *C. difficile* and *C. perfringens* showed an increase in infection rate in phase II compared to phase I as 9.0% to 10.1% and 2.6% to 7.6%, respectively, but these differences were not statistically significant. The changes in the frequency of other pathogens also showed no significant difference.

### The sources of causing bacterial enteritis
The most common cause of bacterial enteritis was unknown (83.2%) (Fig. 4). In cases with a definite causal relationship between enteritis and a source, pork was the most common source (5.6%). The next most common causes were chicken (4.1%), antibiotics (3.0%), and beef (1.0%). With chicken as a source, 6 (75.0%) out of 8 infectious cases were caused by *Campylobacter* enteritis.
The difference of continuous variables based on bacterial species

The mean age of patients infected with *Campylobacter* species was the highest at 125.2±60.5 months compared to the mean age of patients infected with other bacterial species (*p*<0.001) (Table 1). Patients infected with *C. difficile* were the youngest as 44.7±31.5. The mean white blood cell (WBC) was the mean value at the first examination when the patient was hospitalized and *p*-value=0.004. The mean C-reactive protein (CRP) was a mean value at admission and *p*-value=0.001.

### Table 1. The characteristic of continuous variables based on bacterial species

| Bacterial species | Age (mo)* | WBC (×10⁹/mm³)† | CRP (mg/dL)‡ |
|-------------------|-----------|------------------|-------------|
| *Campylobacter*    | 125.2±60.5| 11,227.1±4,176.8 | 6.7±4.8     |
| *Salmonella*       | 68.3±60.3 | 9,727.7±3,434.6  | 5.8±5.4     |
| *Clostridium difficile* | 44.7±31.5 | 13,961.6±8,537.3 | 1.0±1.2     |
| *Shigella*         | 49.6±44.0 | 9,903.6±3,218.7  | 7.0±6.3     |
| *Clostridium perfringens* | 59.0±62.8 | 9,713.6±4,307.2 | 2.9±4.0     |
| *Aeromonas*        | 52.8±39.2 | 11,321.8±3,136.8 | 1.3±1.5     |
| *Yersinia*         | 95.4±29.6 | 15,758.0±8,858.4 | 5.6±3.6     |
| *Verocytotoxin producing Escherichia coli* | 62.2±81.6 | 6,504.0±3,044.9 | 4.2±5.2     |
| *E. coli* O157:H7 | 97.0±55.9 | 8,845.0±2,607.6  | 1.3±1.4     |
| Mean value         | 88.9±64.5 | 10,927.7±4,829.4 | 5.2±5.0     |

Values are presented as mean±standard deviation.

*Age was the mean value presented as months at admission and *p*-value<0.001. †White blood cell (WBC) was the mean value at the first examination when the patient was hospitalized and *p*-value=0.004. ‡C-reactive protein (CRP) was a mean value at admission and *p*-value=0.001.
blood cell (WBC) count at initial examination of patients was 10,927.7±4,829.4/mm$^3$, and patients infected with *Yersinia* species showed the highest WBC count as 15,758.0±8,858.4/mm$^3$ ($p=0.004$). The mean C-reactive protein (CRP) level was 5.2±5.0 mg/dL. The mean CRP of patients infected with *Shigella* species was the highest at 7.0±6.3 mg/dL as compared to patients infected with other organisms ($p<0.001$).

**DISCUSSION**

Infectious diarrhea is a major cause of morbidity and mortality in children [10]. Bacterial pathogens causing infectious diarrhea are deeply associated with contaminated food and water and lead to CAI in children more vulnerable to gastrointestinal infection than adults [11].

The common pathogens causing foodborne bacterial enteritis in the United States are *Campylobacter, Salmonella, STEC, Shigella* and *Salmonella* are the most common causes of death due to diarrhea in all ages worldwide [11,12]. Nontyphoidal *Salmonella* was the most common bacterial enteritis in children from 0–49 years of age in the United States in 2015, followed by *Campylobacter, Shigella*, and STEC [13]. However, *Campylobacter* was the most common pathogen, followed by *Salmonella, Shigella*, and STEC in 2017 [13]. *Campylobacter* has been the common cause of bacterial enteritis since 2010, followed by *Salmonella* species, *C. difficile*, and *Shigella* species. *Shigella* was the third most common pathogen from 2010 to 2015, but infection cases were not identified from 2015 to 2020. However, *Shigella* species are still the major source of bacterial enteritis in developing countries [6]. In Korea, *Shigella flexneri* was a common illness during the Korean War, but since 2005, the incidence of *Shigella* has remained low [14].

*Campylobacter* is a zoonotic pathogen that is the most common cause of bacterial enteritis worldwide [15]. *Campylobacter* was the predominant pathogen that replaced *Salmonella* in Europe in 2005 [16]. These epidemiologic changes may be associated with climate change that impacts food production, supply, distribution, and consumption [5]. Kuhn et al. [15] reported that the number of *Campylobacter* infections significantly increased with increasing temperature and precipitation, so they estimated that Campylobacteriosis would increase by almost 200% in Nordic countries by the end of this century. It has been elucidated by statistical research that change in temperature and precipitation in the Nordic countries is increasing by an average of 4.2°C [15]. We also reported that the number of *Campylobacter* infections increased in phase II (July 2015–June 2020) than in phase I (June 2010–June 2015) in this study. According to data from KDCA on bacterial enteric infection, the most common pathogen from 2015 to 2017 was *Salmonella*, but from 2018, it was changed to *Campylobacter*. The high prevalence of *Campylobacter* infection is due to the low infective dose and an increase in the rate of consumption of chicken meat (according to data from Rural Development Administration in Korea, the annual consumption of chicken per person was 12.8 kg in 2014 to 14.8 kg in 2019) [16]. We presume that the increased intake of chicken meat, an increase in group feeding rates, and climate change may have affected the incidence rate of *Campylobacter* infection in Korea.

In the present study, we explored the epidemiologic changes in bacterial pathogens causing dysentery. We found that the infection rate of *Shigella* decreased, but that of *C. difficile* and *C. perfringens* increased compared to the previous rates. The infection rate of *Shigella* has been declining in the United States and Korea, but cases of multidrug resistance to antibiotics
have been increasing, which is also a public health problem [8,17]. *C. difficile* is reclassified as “*Clostridioides difficile*” and the most common cause of nosocomial infection or healthcare-associated infectious diarrhea [18]. However, asymptomatic carriage and CAI from food, livestock, and other environmental factors has increased to approximately 35% [18]. The CAI of *C. difficile* is mostly caused by the use of antibiotics and proton pump inhibitors (PPIs) and tends to prevail at a younger age than nosocomial or healthcare-associated infections [18]. Hafiz et al. [19] conducted a meta-analysis on the risk of community-acquired enteric infections in PPI therapy. They reported that PPI users showed an increased risk of developing community-acquired enteric infections compared to non-users, and *Salmonella* and *Campylobacter* especially had a stronger association than other bacterial pathogens on the increase of community-acquired enteric infections [19]. Son et al. [20] reported that the rate of *C. difficile* infection has steadily increased from 2006 to 2015 in Korea. A study on *C. difficile* infection in Korean children also showed an increase in the infection rate, and CAI cases showed a higher rate of abdominal pain and hematochezia than those with nosocomial infection [21]. Although there was no statistically significant difference in this study, there was an increase in the rate of *C. difficile* CAIs over the year. These changes in the epidemiology of *C. difficile* may have been caused by the increased use of antibiotics and PPIs in Korean children.

*C. perfringens* form spores, which allows them to be ubiquitous and can produce a large amount of toxins that cause disease in humans and animals [22]. This organism is one of the five pathogens causing foodborne illness in the United States, and one of the causes of bacterial infection outbreaks [22]. *Salmonella*, *Campylobacter*, and *C. perfringens* are the main bacteria that consume contaminated chicken [22]. The consumption of chicken meat is increasing worldwide [22], and it is expected that the infection rate of *C. perfringens* will gradually increase worldwide. In Korea, the incidence of *C. perfringens* infection has increased gradually since 2015.

Along with an increase in *Campylobacter* infections, several population-based studies have been linked to the onset of inflammatory bowel disease (IBD), which has recently increased worldwide [23]. Although the incidence or prevalence of IBD is skyrocketing worldwide as well as in Korea, it is impossible to determine the clear cause of IBD. Chronic inflammation in IBD is well known for excessive mucosal immune responses within the intestinal microflora [24]. *Campylobacter* infection was associated with post-infectious irritable bowel syndrome (IBS), evidenced by an event in Canada in May 2000 [24]. In this case, water contaminated with *E. coli* O157:H7 and *Campylobacter* species from livestock was supplied to people, after which more than 2,300 people showed acute enteritis and 36% of them subsequently developed post-infectious IBS [24]. There IBD and post-infectious IBS are linked with the loss of the intestinal epithelial barrier and failure to downregulate inflammation, leading to intestinal epithelial injury [24]. It was hypothesized that *Campylobacter* infection induced IBD by first disrupting the intestinal epithelial tight junctions and increasing the paracellular permeability of commensal microorganisms such as *E. coli*, thus allowing them to translocate across the intestinal epithelium [24]. Those with an aberrant genetic background, such as a loss of function of NOD2 protein, show defects in bacterial detection and elimination, and an excessive inflammatory response via nuclear factor-κB [24]. However, Nielsen et al. [23] showed that there was no definite correlation between exposure to *Campylobacter jejuni* or *Campylobacter concisus* infection and the development of IBD in a population-based cohort study. A hypothesis about the relationship between the increase in *Campylobacter* infection and the rapidly increasing incidence of
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Pediatric IBD might be possible in Korea, but further study is needed to explain this relationship. Although we did not follow up with all the patients with Campylobacter infection, there were no patients that developed IBD after Campylobacter-induced colitis, among the included patients in this study.

*C. jejuni* among Campylobacter species is the most common pathogenic species in human infections [25]. Cases of campylobacteriosis are very closely related to the consumption of food or drinks such as poultry, water, and contaminated unpasteurized milk [15]. The infection rate was highest in the late spring and summer seasons [15]. A study on adult patients reported that there were two peak incidences of Campylobacter infection as in younger adults (16–25 years) and older patients than 75 years [26]. In our study showed the mean age of patients with Campylobacter infection was 125.2±60.5 months (range: 0–208 months). This was the highest mean age compared to other pathogens leading to CABE.

The most common symptoms of Campylobacter infection include colicky abdominal pain, acute diarrhea, and fever [27]. In this study, fever and diarrhea were the most common symptoms seen in 92.9% of patients and 54.8% of patients showed severe diarrhea symptoms that persisted even during sleep. Jang et al. [27] reported that diarrhea was the most common symptom (97.6%), followed by fever (96.7%) and abdominal pain (94.3%) in a single-center study of Campylobacter enteritis. Laboratory findings of Campylobacter infection in CRP was 6.70±4.8 mg/dL in this study. This was the second highest level observed after *Shigella* infection.

In this study, elevated levels of CRP have been seen in school-age children or adolescents with fever, or abdominal pain since 2015. Based upon these findings, Campylobacter should be investigated as the primary causative pathogen, before considering other pathogens. Patients with suspected bacterial enteritis with dysenteric symptoms such as diarrhea, cholera infection with severe dehydration, premature infants, and immunocompromised children should consider empirical antibiotics [28]. Most Campylobacter enteritis cases are self-limiting, but in severe cases showing prolonged fever with severe abdominal pain or high level of CRP with persistent bloody diarrhea and immune deficiency, or in case of infected infants, appropriate empirical antibiotics should be administered early on [28]. Third-generation cephalosporins are the drug of choice for *Salmonella typhi* and *Shigella* infections [29]. Ceftriaxone was prescribed to hospitalized patients, and cefixime was used in outpatient cases with these infections [30]. However, *Shigella* and *Salmonella* species are resistant to third-generation cephalosporins, and if the patient did not show clinical improvement, azithromycin was replaced as the second antibiotic. Macrolides are the most commonly used antibiotics for Campylobacter infections that require antibiotic treatment [31]. Specifically macrolides and fluoroquinolones are the most commonly used antibiotics for Campylobacter-induced enteritis [31]. Macrolides are preferable to fluoroquinolones in children because of their low resistance rate and safety in an environment of high antibiotic resistance for enteric bacteria [31]. Among macrolides, azithromycin is the first choice for Campylobacter infection and can also be used for *Vibrio cholerae*, *Aeromonas*, *Yersinia*, and *Shigella* infections [31]. We also used ceftriaxone as the first-line treatment for CABE in our center. Therefore, we could consider azithromycin instead of ceftriaxone as the first empirical antibiotic in Korean children with indications for antibiotic use due to CABE.

This study had several limitations. First, it was a single-center study, so we cannot generalize these results as a general situation in Korean children. Second, epidemiologic data from this
study cannot be reflected in the data of all Korean children because some pathogens that could not be identified in the PCR kit used in this study were excluded from the results. Third, the possibility of false-positive results for pathogens cannot be completely excluded because we did not perform culture studies for pathogens. However, despite these limitations, this study has the value of rare research on CABE in children only. We believe that this study will be helpful in recognizing the epidemiological changes in CABE in Korean children and establishing treatment policies, such as empirical antibiotics, through this study.

We should also make efforts to identify the pathogens of CABE outbreaks based on the results obtained from continuous large-scale epidemiological studies and to establish health and medical policies to decrease CABE in Korea.

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