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Enteric Pathogens in Pediatric Patients with Acute Gastroenteritis in Gyeongju, Korea: A Retrospective Study for 7 Years in a Regional Hospital

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Acute diarrhea is a global health problem that causes high morbidity and mortality in children. Notably, enteric pathogen co-infections have been suggested to play an important role in gastroenteritis. In this study, we analyzed 1150 stool specimens of patients who visited the pediatric ward of Dongguk University Hospital in Gyeongju province from January 2011 to December 2017. The average isolation rate of potential stool pathogens over 7 years was 37.3% (429/1150), and coinfections were observed in 51 patients (51/429; 11.9%). In the 51 co-infection cases, the most frequent type of co-infection was found to be that of virus-bacteria (33/51). The most frequently detected bacterial pathogen among the co-infected cases was *Clostridium* spp. (22/51), out of which *Clostridium perfringens* was found to be the main pathogen (16/22; 72.7%). *Escherichia coli* spp. were the second most common bacterial pathogens found in 12 cases (12/51; 23.5%), with 10 cases of *E. coli* EPEC. Furthermore, the most frequently implicated viral pathogen among the co-infected cases was norovirus (16/51), followed by rotavirus (12/51).

Keywords: **Enteric stool pathogen, coinfection, *Clostridium perfringens*, norovirus**

Introduction

Diarrhea accounts for 4.1% of all disease worldwide [1] and is caused by variety of enteric viruses, bacterial pathogens, and parasites. The clinical epidemiology of viral gastroenteritis, including the prevalence and vulnerable age, has changed since the introduction of rotavirus vaccination [2]. Coinfection with multiple pathogens may be common among children with diarrhea and causes more severe diarrhea than infection with a single pathogen [3]. However, few studies have examined coinfections in Korean children with watery diarrhea after the introduction of rotavirus vaccination. Thus, the aim of this study was to validate the trend of coinfections in Korean children with acute gastroenteritis (AGE), including the age and seasonal distribution, in this population. To determine the trends in bacterial and viral species as possible pathogenic microorganisms causing pediatric diarrhea in Gyeongju province, bacterial and viral pathogens isolated by year, season, and age were analyzed from 2011 to 2017.

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Materials and Methods

Patients and samples

A total of 1,150 clinical stool specimens were collected from patients with diarrhea who visited the pediatric ward of Dongguk University Hospital in Gyeongju

province, South Korea, from January 2011 to December 2017. This study was approved by the Institutional Review Board of Dongguk University Gyeongju Hospital (number: 110757-201810-HR-03-03).

Microbiological methods

Enteric bacterial pathogens were identified using automated equipment (Vitek®2 compact, bioMérieux, Marcy-lÉtoile, France) after incubating stool specimens from children with diarrhea. If the isolated strains were identified as *Salmonella*, *Shigella*, or *Escherichia coli*, an additional antiserum test (Joongkyeom Co., Korea) was conducted.

As part of the Laboratory Surveillance of Acute Gastroenteritis, the stool specimens were sent to the Gyeongsangbuk-do Institute for Health & Environment to identify the bacterial and viral pathogens. The Institute for Health and Environment diluted 100 µl diarrheal stool with 900 µl PBS to detect diarrhea-causing bacteria followed by cultivation for 4 h at 37℃ in tryptic soy broth (Oxoid, Ltd., UK). After cultivation, centrifugation was conducted at 9,000 rpm for 3 min; 5 µl of the supernatant was used for PCR (C1000 Touch™ Thermal Cycler, Bio-Rad, USA) using PCK kits (Kogenebioteck Co., Korea) to detect gram-positive bacteria (PowerChek™ Gram Positive Multiplex detection Kit), gram-negative bacteria (PowerChek™ Gram Negative Multiplex Detection Kit), and *E. coli* (PowerChek™ Diarrhea *E. coli* 8-plex Detection Kit). The amplified PCR products were verified with a MuliNA Electrophoresis system (MCE-202, Shimadzu Biotech, Japan).

Stool specimens showing positive results by PCR were cultured using standard methods. To identify enteric bacteria such as *Salmonella*, *Shigella*, *E. coli*, *Yersinia*, *Campylobacter*, *Vibrio*, and *Listeria*, samples were inoculated into appropriate selective medium after enrichment. Other bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens* were directly inoculated into selective medium. All inoculated selective media were cultivated under optimal conditions at an appropriate temperature, oxygen concentration, and incubation time. Separated bacterial colonies were identified using a Vitek®2 compact system (bioMérieux).

To identify the enteric viral stool pathogens, the Institute for Health and Environment diluted 100 µl of diarrheal stool with 900 µl PBS followed by centrifugation at 13,000 rpm for 3 min to obtain the supernatant. To identify norovirus, astrovirus, and sapovirus, nucleic acids were extracted from 150 µl of supernatant using nucleic acid extractor (Freedom EVO-2 100 base, Tecan Schweiz AG, Männedorf, Switzerland). Next, 5 ml of extracted viral nucleic acid was amplified with a 7500 Fast Real-Time PCR System (Applied Biosystems, USA) using a PowerCheck Real-time PCR kit (Kogenebioteck Co., Korea). To detect adenovirus and rotavirus, 100 µl of supernatant was analyzed by enzyme-linked immunosorbent assay using a RIDASCREEN test kit (R-biopharm, Germany).

Results

Clinical characteristics of subjects and distribution of enteric pathogens

The average of isolation rate of potential stool pathogens over the 7 years was 37.3% (429/1150); of the 429

Table 1. Distribution of patients according to the age group for 7 years.

Year	No. of patients	Age (month)			No.(%) of patients with pathogen	Age (month)		
		<12	12-59	≥60		<12	12-59	≥60
2011	132	54	54	24	50 (37.9)	11 (20.4)	29 (53.7)	10 (41.7)
2012	81	30	27	24	29 (35.8)	10 (33.3)	11 (40.7)	8 (33.3)
2013	88	37	26	25	27 (30.7)	5 (13.5)	11 (42.3)	11 (44.0)
2014	155	52	57	46	51 (32.9)	16 (30.8)	16 (28.1)	19 (41.3)
2015	134	43	44	47	42 (31.3)	14 (32.6)	14 (31.8)	14 (29.8)
2016	188	40	78	70	74 (39.4)	9 (22.5)	32 (41.0)	33 (47.1)
2017	372	91	155	126	156 (41.9)	32 (35.2)	70 (45.2)	54 (42.9)
Total	1150	347	441	362	429 (37.3)	97 (28.0)	183 (41.5)	149 (41.2)

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Table 2. Summary of isolated stool pathogens from children with AGE from 2011 to 2017 in a regional hospital.

Year / No of samples	2011	2012	2013	2014	2015	2016	2017	Total
all stools	132	81	88	155	134	188	372	1,150
all isolates	50	29	27	51	42	74	156	429
Bacterial isolates	16	6	15	31	23	43	89	
Salmonella spp.	16	5	7	10	9	9	20	76
Clostridium spp.	0	0	0	0	0	21	41	62
S.aureus	0	0	2	16	9	0	0	27
E. coli	0	1	6	5	5	8	12	37
Campylobacter spp.	0	0	0	0	0	3	8	11
Other spp.*	0	0	0	0	0	2	8	10
Viral isolates	37	25	14	25	23	38	92	
Norovirus	10	17	4	16	11	26	33	117
Rotavirus	21	3	5	6	9	3	37	84
Adenovirus	5	3	3	1	1	4	13	30
Astrovirus	1	2	1	1	1	5	7	18
Sapovirus	0	0	1	1	1	0	2	5
Mixed isolates	2	1	2	6	5	10	25	51

**Vibrio* sp. 1 on 2016; *Yersinia enterocolitica* 4 on 2017; *Aeromonas* spp. 1 on 2016 and 4 on 2017.

children evaluated, 280 (69.5%) were under 5 years of age and 97 (28%) were under 1 year of age (Table 1). The percentages of viral and bacterial infections were 52.6% and 47.4%, respectively. Among the virus pathogens, norovirus was the most frequently identified pathogen, followed by rotavirus. Among bacterial pathogen, *Salmonella* was the most common, followed by *Clostridium species*, *E. coli*, *S. aureus*, and *Campylobacter* spp. (Table 2).

Distribution of coinfected enteric pathogens

Confections were observed in 51 patients (51/429 = 11.9%). Of the 51 cases with coinfections, the most frequent type was virus-bacteria coinfection (33/51) (Table 3). The most frequently implicated bacterial pathogen among co-infected cases was *Clostridium* spp. (22/51). Among the *Clostridium* spp., *C. perfringens* was the main pathogen (16/22 = 72.7%), followed by *Clostridium difficile* toxin B (5/22 = 22.7%). The second most common type was mixed bacterial coinfection, which was found in 11 cases (11/51 = 21.5%). A total of 64% of cases of mixed bacterial coinfection was found in children over 5 years old. *Escherichia coli* spp. was the second most common bacterial pathogen and was found in 12 cases (12/51 = 23.5%), among which 10 were *E. coli* EPEC. *Salmonella* spp. was found only 1 case and other bacterial pathogens included *S. aureus*, *Vibrio* spp., *Campylobacter* spp., *Bacillus* spp., *Aeromonas* spp., and *Y. enterocolitica.*

The most frequently implicated viral pathogen among co-infected cases was norovirus (16/51), followed by rotavirus (12/51). For adenovirus, 80% of adenoviral coinfection (4/5) was found in children younger than 5 years.

Seasonal prevalence of enteritis pathogen

Salmonella spp. infection gradually increased in the spring and peaked in the summer (from July to September), followed by drastic decreases in the fall and winter. *Escherichia coli* increased during the summer months, particularly in July and August (Fig. 1A). In contrast, distinct seasonality was observed in norovirus infection. Norovirus infection increased from October and reached a peak in December, followed by a decline until March. Rotavirus infection gradually increased from October and reached a peak in April, and then decreased until September. In contrast, infections with adenovirus, astrovirus, and sapovirus showed similar levels throughout the year (Fig. 1B).

Table 3. Distribution of enteric pathogens in coinfections according to the age group from 2011 to 2017.

Subjects of pathogens*	No of Age group (month)					
	<12		12-59		≥60	
Viral coinfection	1		5		1	
	1	RotaV + AdenoV	3	NoroV + RotaV	1	RotaV + NoroV
			1	RotaV + AdenoV		
			1	NoroV + AstroV		
Viral and bacterial coinfection	10		13		10	
	2	*C. perfringens* + RotaV	3	*C. perfringens* + NoroV	2	*S. aureus* + NoroV
	2	*E. coli* EPEC + NoroV	1	*C. difficile* toxin B + *C. perfringens* + NoroV	2	*C. perfringens*+ NoroV
	2	*S.aureus* + NoroV	1	*E. coli* EPEC + *S. aureus* + RotaV	1	*C. perfringens* + RotaV
	1	*C. perfringens* + NoroV	1	*E. coli* EPEC + AdenoV	1	*C. difficile*toxin B + NoroV
	1	*S. aureus* + AstroV	1	*E. coli* EPEC + NoroV	1	*E. coli* EPEC + RotaV
	1	*C. difficile* toxin B + SapoV	1	*S. aureus* + AdenoV	1	*E. coli* EAEC + NoroV
	1	*E. coli* EPEC + *S. aureus* + NoroV	1	*E. coli* EPEC + RotaV	1	*Bacillus* + AstroV
			1	*Bacillus* + RotaV	1	*C. perfringens* + AdenoV
			1	*Clostridium* + NoroV		
			1	*C. difficile* toxin B + NoroV		
			1	*C. perfringens* + RotaV		
Bacterial coinfection	2		2		7	
	1	*C. perfringens* + *Aeromonas* spp.	1	*Campylobacter* spp. + *C. perfringens*	1	*E. coli* O157:H7 + *C. perfringens*
	1	*E. coli* VTEC + *C. perfringens*	1	*Y. enterocolitica* + *Aeromonas* spp.	1	*Y. enterocolitica* + *C. perfringens*
					1	*E. coli* EPEC + *S. aureus*
					1	*C. perfringens* + *Campylobacter* spp.
					1	*C. difficile*toxin B +*C. perfringens*
					1	*Salmonella(S. albany)* + *E. coli* EPEC
					1	*Vibrio* spp. + *Y. enterocolitica* + *C. perfringens*

*Abbreviations: NoroV, Norovirus; AdenoV, Adenovirus; RotaV, Rotavirus; AstroV, Astrovirus; SapoV, Sapovirus.

Age-specific pattern of pediatric diarrheal pathogen

Salmonella spp. was the most common pathogen in children over 2 years old. Among children under 1 year old, *Clostridium* spp. was most common pathogen, followed by *S. aureus* (Fig. 2A). For viral pathogens, norovirus was the most common viral pathogen in all age groups except for 4 years old. For patients who were 4 years old, rotavirus was the most common causative virus, followed by norovirus. Adenovirus was mainly found in the age group below 6 years old 28 out of 30 patients (Fig. 2B).

Discussion

In our study, evidence of infection was found in most AGE cases. Among the bacterial pathogens (n = 231), the prevalence of *Salmonella* spp. was highest (n = 76, 32.9%), followed by *Clostridium* spp. (n = 62, 26.8%) and *E. coli* spp. (n = 37, 16.0%). Eleven cases of *Campylobacter* spp. were detected (4.8%). Of the 51 coinfections, *Campylobacter* spp. was found in only one case, which differs from the previous studies [4, 5] reporting that incidence of *Campylobacter* spp. was the most common followed by *Salmonella* spp. and *C. perfringens*. According to the study published in 2008 [6], the three major causes of diarrhea in Korea were pathogenic *E. coli*, *S. aureus*, and *Salmonella* species. Other related pathogens were *B. cereus*, *V. parahaemolyticus*, *C. jejuni*, *C. perfringens*, and *Shigella*. In the study [6] that included adult, adolescents, and children, *Campylobacter* spp.

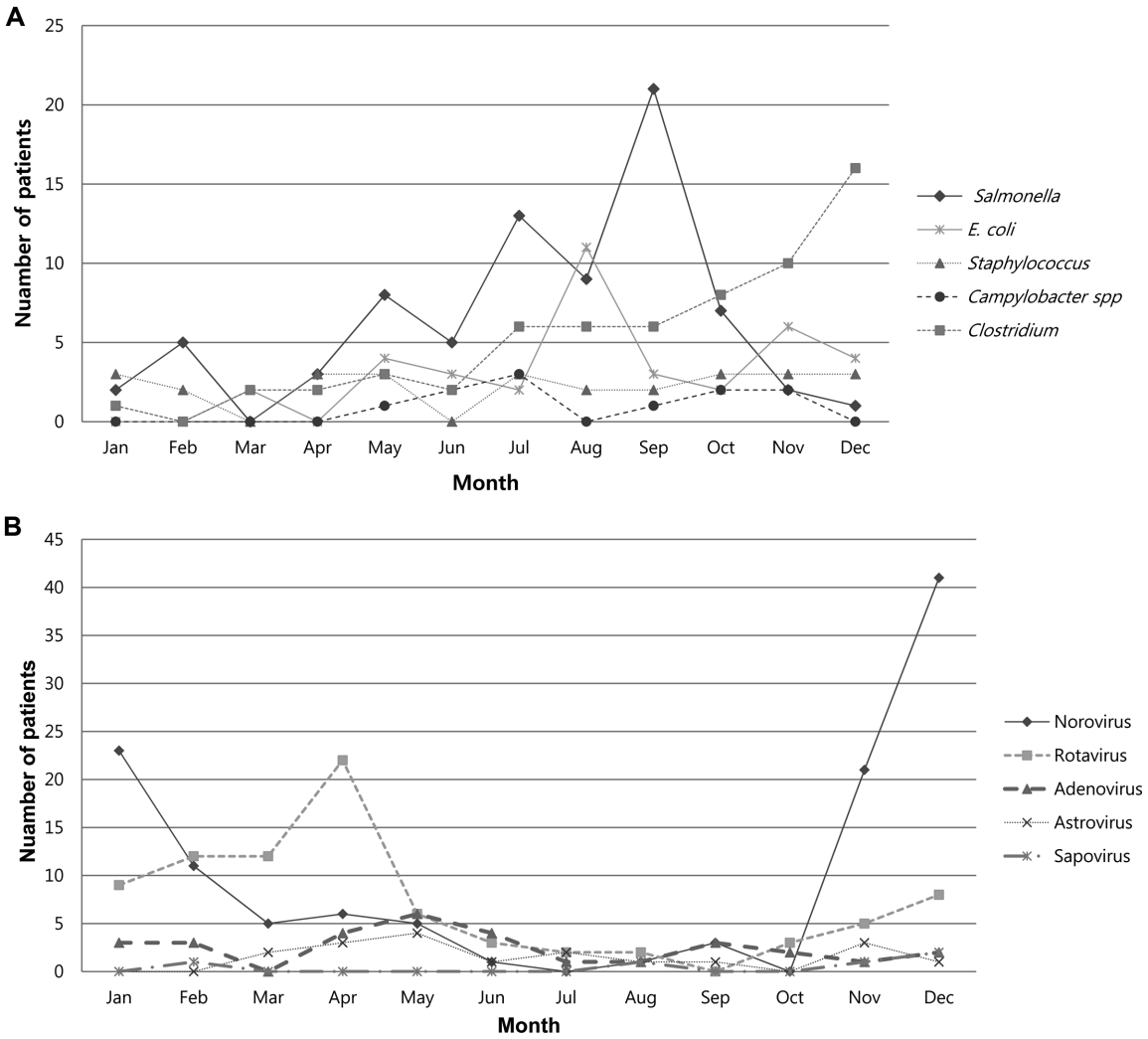

Fig. 1. (A) Monthly distributional trends in the major proportions of bacterial organisms among 429 isolates from 2011 to 2017, (B) Monthly distributional trends in the proportions of viral organisms among 429 isolates from 2011 to 2017.

was not the main stool pathogen of acute enteritis approximately 10 years ago. Thus, the main causative pathogens of acute inflammatory diarrhea have changed over last decade. The different figures observed in other districts may be explained by the different age groups, seasonal variations at the time of sampling, and detection methods used.

Coinfections were observed in 11.9% of cases and the most common type was virus-bacteria coinfection (33/51). According to previously published studies, virus-virus coinfection was the most common type [7] and among the viral pathogens, rotavirus was the viral agent most frequently implicated in coinfections [7, 8]. Another study of viral coinfection published in 2008 [9] reported that rotavirus and norovirus were the most common co-infectious agents responsible for acute watery diarrhea in Korean children. In cases of virus-bacteria coinfection, a recent study showed that coinfections with rotavirus and *C. difficile* were the most common [10]. However, norovirus coinfection has recently become the most prevalent type according to a few studies conducted in the era of rotavirus vaccination [11, 12]; we also showed that norovirus infection was the leading cause of AGE in hospitalized children, exceeding rotavirus infection, and the most frequently implicated viral and bacterial pathogens of coinfection were norovirus (16/51) and *C. perfrin-*

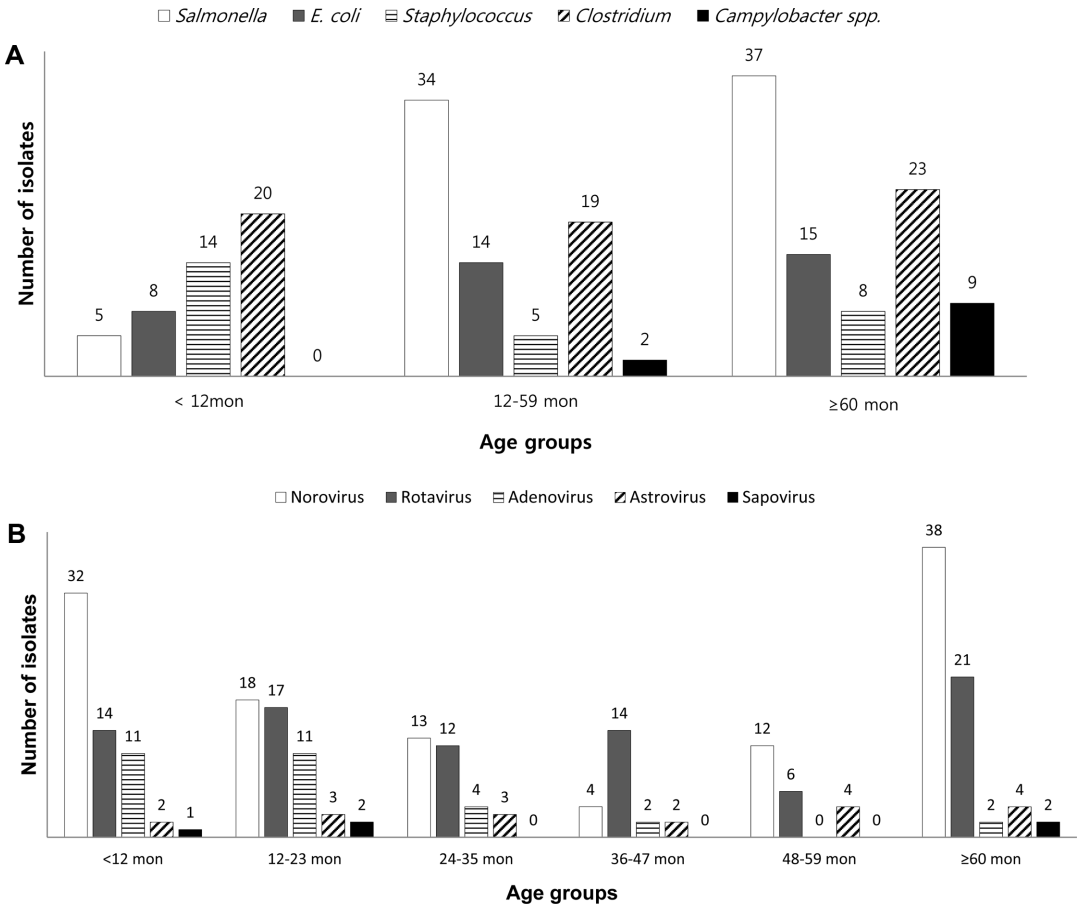

Fig. 2. (A) Distribution of the five most prevalent bacterial organisms from 2011 to 2017 according to the age group, (B) Distribution of viral organisms from 2011 to 2017 according to the age group.

gens (16/51), respectively. Viral and bacterial intestinal pathogens may affect either the same or different regions of the gut and show enhanced effects [13]. However, the pathophysiology of *C. perfringens* in the human gut as a coinfection source with virus is not well-understood.

Rotavirus began increasing in the winter and continued to be detected until late spring (Fig. 1B). The influence of vaccination on seasonality of infection previously appeared to be minimal. Studies conducted before and after the introduction of rotavirus vaccine [14, 15] showed similar seasonality of rotavirus infection compared to this study. However, changes in seasonality may have occurred because of the influx of new types of rotaviruses or through genetic changes in rotavirus after vaccine introduction. In contrast, norovirus increased rapidly in the winter, but showed a sharp decline in the spring. Previous studies reported that the peak period of norovirus infection is November [16, 17] and a recent study reported that norovirus infection occurs mainly in the winter season, peaking from November to January [18].

The adenovirus-positive rate in children with diarrhea was 2.6% (30/1150 samples). Previous studies reported that the prevalence rate of adenovirus ranged from 2% to 23.2% [19–21]. Adenovirus was mainly found in children under 5 years of age in this study. Twenty-eight of the 30 patients (93%) were under 5 years old, which is similar to previously reported results in Korea [9]. According to a study conducted in Japan and Vietnam [22], 83 of 100 and 37 of 38 patients, respectively, were under 36 months old. For coinfections, adenovirus was found in 5 cases (5/51) and 80% of adenoviral coinfection (4/5) was found in children younger than 5 years. Breast-

feeding, socioeconomic status, hygiene, and climate may make children under 5 years of age more vulnerable to adenovirus infection.

There were some limitations to our study. This study was conducted in a single research institute, and thus the number of cases was relatively small. There were difficulties in interpreting the results because of the retrospective nature of the study. In conclusion, this study revealed the changing prevalence of bacterial and viral infection in AGE and the pattern of coinfections after introduction of rotavirus vaccination in Gyeongju province. The most common type of coinfection was virus-bacteria coinfection. *Salmonella* spp. was the main causative bacterial pathogen other than *Campylobacter* spp., and norovirus was the leading cause of diarrhea-causing enteritis in hospitalized children, exceeding the rate of rotavirus infection.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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