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Using Multiplex Molecular Testing to Determine the Etiology of Acute Gastroenteritis in Children

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Abstract

Objective—To detect the etiologic agents of acute gastroenteritis (AGE) in children using broad molecular-based techniques, and compare clinical presentations among etiologies.

Study design—This was a prospective population-based surveillance study of children aged <6 years with AGE conducted between 2008 and 2011 as part of the New Vaccine Surveillance Network. Stools from patients and healthy controls were tested for 21 gastrointestinal pathogens using the analyte-specific reagent Gastrointestinal Pathogen Panel and an additional reverse transcription real-time polymerase chain reaction assay for sapovirus and astrovirus.

Results—Of the 216 stool samples from patients with AGE, 152 (70.4%) tested positive for a pathogen, with norovirus genogroup II (n = 78; 36.1%) and *Clostridium difficile* (n = 35; 16.2%) the most common pathogens detected. Forty-nine patients (22.7%) tested positive for more than 1 pathogen, including 25 (71%) with a *C difficile* detection. There were no significant clinical differences among the patients with no pathogen detected, those with a single pathogen detected, and those with ≥2 pathogens detected.

Conclusion—Using a broad molecular testing approach, high rates of enteropathogens were detected in children with AGE, dominated by norovirus genogroup II and *C difficile*. Coinfections

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were common but had no identifiable impact on clinical manifestations. As routine diagnostics of AGE progressively evolve toward nucleic acid–based pathogen detection, ongoing systematic studies are needed to better analyze the clinical significance of results.

Acute gastroenteritis (AGE) is one of the most common infectious diseases of humans, with approximately 5 billion episodes of diarrhea occurring worldwide annually, accounting for 15%-30% of all deaths in some countries.[1] Viral AGE accounts for approximately 3%-5% of all hospital days and 7%-10% of hospitalizations of children each year in the US.[1,2] Norovirus (a member of the family *Caliciviridae*) has become the leading cause of gastroenteritis among young US children since the implementation of universal rotavirus immunization of infants beginning in 2006.[3] In addition, the rates of *Clostridium difficile* infection (CDI) in children are on the rise. A recent national database study of hospitalized children found an increase in incidence from 24 to 58 per 10 000 discharges per year between 2003 and 2012.[4]

In a large proportion of patients, the etiologic agent of AGE is unidentified. In 2009-2010, a total of 2259 AGE outbreaks were reported to the Centers for Disease Control and Prevention (CDC).[5] No etiologic agent was identified in samples from approximately 40% of these outbreaks, underscoring the need to broaden testing for additional enteric pathogens. Recent work has demonstrated that polymerase chain reaction (PCR)-based testing has better sensitivity than conventional methods of enteropathogen detection[6-8]; however, the time and materials required to test for each of the common causes of AGE individually makes this approach impractical for routine clinical application. In addition, concerns remain that for certain pathogens (*Clostridium difficile* in particular), the superior sensitivity of nucleic acid–based testing creates the potential for overdiagnosis and overtreatment.[9] Finally, the identification of some pathogens in asymptomatic control children makes the interpretation of their detection in symptomatic patients more problematic.

Several multiplex nucleic acid–based strategies to test for multiple gastrointestinal pathogens have been devised to surmount the diagnostic challenges inherent to the causal diversity of AGE.[10-14] The recently developed Gastrointestinal Pathogen Panel (GPP; Luminex Molecular Diagnostics, Toronto, Ontario, Canada) allows the simultaneous detection of 19 unique gastrointestinal pathogen sequence targets in fecal specimens using multiplex PCR amplification.[15]

In this study, we used the GPP and an additional multiplex reverse-transcription real-time PCR (RT-rtPCR) analysis for the detection of sapovirus and astrovirus to identify enteropathogens in fecal specimens from children aged 2 weeks to 6 years who were prospectively enrolled in a multiyear surveillance project for AGE at the study site in Nashville, Tennessee. In addition, we tested samples from healthy asymptomatic children and a subgroup of patients with known rotavirus gastroenteritis to determine the degree of positive testing in these 2 unique control cohorts.

Methods

The study population comprised children living in Davidson County, Tennessee who received care at Monroe Carell Jr Children's Hospital at Vanderbilt (MCJCHV) between May 1, 2008, and June 30, 2011, excluding October 2009. The age at study enrollment ranged between 14 days and <5 years through October 31, 2010, and between 14 days and <6 years from November 1, 2010, through completion of the study. Prospective, active surveillance of children occurred as part of the New Vaccine Surveillance Network (NVSN).[16] Institutional Review Board approval for the study was obtained from Vanderbilt University and the Centers for Disease Control and Prevention.

Children with ≥3 loose stools and/or ≥1 episode of vomiting within a 24-hour period and presenting within 10 days of illness onset were eligible for enrollment. Children seen in the inpatient, emergency department, and outpatient settings were included. Children who likely had noninfectious diarrhea, had a known clinical immunodeficiency, had been previously enrolled for the same gastroenteritis episode, or had been transferred from another institution after >48 hours of hospitalization were excluded from the study. Stool specimens, provided almost exclusively in the form of soiled diapers, were collected at the time of enrollment or as soon as possible within 14 days of AGE symptom onset.

Healthy controls were children aged <5 years[17] enrolled between May 1, 2009, and August 31, 2009, at the time of a well-child visit at MCJCHV. These children had no known clinical immunodeficiency, no symptoms of an acute respiratory infection (ie, cough, congestion, sore throat, wheezing, or rhinorrhea) within 3 days of enrollment, and no symptoms of AGE within 14 days of enrollment.

Specimens from an additional subset of children with AGE enrolled in the NVSN between 2006 and 2009 and who tested positive for rotavirus by enzyme immunoassay (EIA) (Rotaclone; Meridian Bioscience, Cincinnati, Ohio) were also evaluated for additional pathogens using the methods described above.

Demographic, epidemiologic, and clinical data were collected for all subjects, as were provider-verified rotavirus vaccination records.

Specimen Processing

Specimens were refrigerated or frozen within 8 hours of collection and stored at −20°C for approximately 2 weeks until processing. Stool suspensions (10%) were prepared in Rotaclone EIA diluent or phosphate-buffered saline and tested immediately for the presence of rotavirus in accordance with manufacturer's instructions. Scant or liquid specimens insufficient for testing were recovered using a plastic syringe barrel and plunger to forcefully pass 10 mL of Earl's balanced salt solution through an excised piece of soiled liner, resulting in a fecal eluate. Residual clarified stool suspensions were stored at −80°C.

Nucleic Acid Extraction

Total nucleic acid was extracted from 10% clarified stool suspensions by preliminary mechanical disruption using Presells Soil Mix Beads (Cayman Chemical, Ann Arbor,

Michigan), followed by purification using the Magma Total Nucleic Acid Isolation Kit (Applied Biosystems, Carlsbad, California) and Magma Express 96 (Applied Biosystems) automated extraction system in accordance with the manufacturer's instructions. Before extraction, each specimen was seeded with 10 μL of MS2 bacteriophage lysate (ZeptoMetrix, Franklin, Massachusetts) at 1×10^9 PFU/mL as a processing and amplification control to confirm the success of nucleic acid extraction and the absence of endogenous PCR inhibitors.

Enteropathogen Detection

The gastrointestinal pathogens included in our analysis are summarized in Table I. In addition, we also tested for astrovirus and sapovirus using a multiplex reverse-transcription quantitative polymerase chain reaction assay on an Applied Biosystems 7500 Real-Time PCR platform. MS2 was concurrently amplified in a separate reaction according to published methods.[17] GPP testing was performed as described previously with some modifications to the PCR master mix and analysis conditions.[15] In brief, each PCR reaction contained 0.167 μL of each ASR (Luminex Corporation, Austin, Texas), 7.5 μL of 5× RT-PCR buffer (Qiagen, Valencia, California), 1.45 μL of 10 mM deoxyribonucleotide triphosphate mixture (Qiagen), 0.5 μL of bovine serum albumin (New England Biolabs, Ipswich, Massachusetts), 0.1 μL of 5M tetramethylammonium chloride (Sigma-Aldrich, St Louis, Missouri), 2 μL of OneStep RT-PCR enzyme (Qiagen), and 0.11 μL of DNAse/RNAse free water. The total reaction volume was 25 μL (15 μL of master mix plus 10 μL of extracted nucleic acids). Target-specific PCR products were detected using Luminex xMAP technology with fluorescent microspheres and the Luminex 100/200 microfluidics system, with gating set at 7000-20 000 according to the Luminex protocol. Data were analyzed using xPONENT 3.1 and TDAS LSM software packages (Luminex Corporation, Austin, Texas). MS2 sequences were coamplified in the same reaction tube with xTAG targets. Determination of a positive specimen is detailed in Table II (available at www.jpeds.com).

Statistical Analyses

The Pearson χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables were used for comparisons of demographic, epidemiologic, and clinical characteristics, with a 2-sided P value < .05 considered to indicate statistical significance. In the event of a statistically significant finding using the Kruskal-Wallis test, additional pairwise comparisons were performed using the Wilcoxon rank-sum test with Bonferroni correction to adjust for multiple comparisons.

Results

The group of 216 children with AGE included 99 females (46%) and 117 males (54%) and had a median age of 16 months (IQR, 6-27 months). Seventeen patients (8%) had a history of prematurity; 150 patients (69%) had a history of breast-feeding, and 23 (11%) were being breast-fed at the time of study enrollment. One or more enteropathogens were detected in stools from 152 of the 216 children with AGE (70.4%) enrolled during study years 2008-2011, vs 4 of the 36 asymptomatic control patients (11%) enrolled in 2009. A viral pathogen was present in 138 children in the AGE group (63.9%) compared with only 1 child

in the control group (3%), whereas a bacterial pathogen of AGE was detected in 47 cases (21.8%) vs 3 controls (8%). Episodes of detection in asymptomatic children included 1 norovirus GII and 3 *C difficile*. Among the 21 asymptomatic infants aged <12 months, 3 (14%) tested positive for *C difficile*.

Of the 138 samples from patients with AGE cases who tested positive for a virus, norovirus GII was detected in 78 cases (57%) and rotavirus was detected in 28 cases (20%) (Figure 1). *C difficile* was identified in 35 (75%) of all samples that tested positive for enteric bacteria. All specimens tested negative for protozoan parasites. Forty-nine patients with AGE (22.7% of the total cohort) tested positive for more than 1 pathogen. Of these, 7 patients had 3 pathogens detected. Of the 35 patients who tested positive for *C difficile*, 25 (71%) also tested positive for at least 1 other pathogen. The most common combination of pathogens was norovirus GII and *C difficile* (Figure 2). *C difficile* was more prevalent in children aged <3 years, with 94% of *C difficile* detections in these young children. More patients were enrolled during the fall and winter months, more than one-half of whom had a viral etiology (Figure 3; available at www.jpeds.com). In contrast to the nominal rotavirus positivity among AGE patients enrolled during the 2009-2010 winter and spring seasons, in which rotavirus was detected in only 2% of patients, 31% of AGE cases enrolled during the 2010-2011 winter and spring seasons were rotavirus-positive. This marked fluctuation in rotavirus prevalence corresponds with a roughly biennial epidemic pattern of rotavirus in the US that has emerged in the postvaccine era.[18]

In patients with AGE, comparisons of those with a single viral pathogen, a single bacterial pathogen, coinfection with more than 1 pathogen, and no pathogen detected based on demographic and clinical characteristics (Table III), no statistically significant differences in race or sex were identified. However, there were statistically significant differences in age (P = .04), number of hospital days in those patients who required admission (P = .002), and maximum number of diarrhea episodes in 24 hours (P = .015), but these findings did not persist after pairwise comparisons were completed and a Bonferroni correction was applied. Patients with a single viral pathogen identified were more likely to experience vomiting (91%), whereas those with a single bacterial pathogen identified were more likely to have fever (85%), but these findings were not statistically significant. Asymptomatic control patients were also compared on demographic characteristics, and there were no statistically significant differences in terms of age, race, or gender when comparing patients with a bacterial detection, viral detection, or no pathogen identified.

Stools from 64 children with EIA-confirmed rotavirus AGE in 2006-2009 were included in the analysis as a reference for the frequency and spectrum of copathogens in the presence of a clear cause for AGE. Additional enteropathogens identified in this rotavirus-positive population included *C difficile* in 11 patients (17%) and *Campylobacter* in 1 patient (2%). In this cohort, the *C difficile*–positive children were younger than the *C difficile*–negative children, but the difference was not statistically significant (14.8 months vs 18.0 months; P = .39, Wilcoxon rank-sum test).

Discussion

Despite the substantial health burden of pediatric AGE, the etiologic profile of AGE in children remains poorly defined. Newer technologies, such as multiplex molecular assays, allow for rapid and sensitive analysis of AGE pathogens and are now commercially available and Food and Drug Administration–cleared. Previous studies have validated the analytical sensitivity and specificity of these methods, but few have addressed the clinical significance of the results.[10-13]

Using multitarget molecular assays, our study identified enteropathogens in 70.4% of our cohort of children aged 14 days through 6 years with symptomatic AGE. This is in stark contrast to a previous study using primarily nonmolecular methods such as culture, microscopy, and cytotoxicity assays to identify pathogens in symptomatic children with AGE.[19] Using these traditional approaches, a pathogen was detected in only 29 of 254 ill children (11.4%), illustrating the superior sensitivity of molecular as-says. In the present study, norovirus GII was the most common infection identified, with 36.1% of stools testing positive. Since implementation of routine rotavirus vaccination in the United States, norovirus GII has emerged as the predominant cause of AGE among young children.[17] Norovirus was detected in 21% of 1295 children seeking medical attention for AGE in 2009-2010 (including 20 of 57 [35%] outpatients evaluated at MCJCHV), whereas rotavirus was identified in only 12% of these patients.[3] The high rate of norovirus in this population supports the need for an effective norovirus vaccine, as well as appropriate norovirus infection control measures.

Pediatric CDI is on the rise in both inpatient and outpatient settings.[20-23] *C difficile* was the most common bacterial infection identified in our cohort, present in 16.2% of stools of children with AGE. Although *C difficile* is a well-established cause of AGE in children, it is also detected in 25%-80% of infants aged <1 year without AGE symptoms, with lower colonization rates in toddlers.[24-26] High rates of colonization in children with comorbidities and/or frequent hospitalizations also have been reported, with rates as high as 30%-55% in pediatric oncology patients, 17% in children with inflammatory bowel disease, and 24% in hospitalized children.[27-29] In the present study, we found *C difficile* colonization in 8% of healthy asymptomatic children aged 0-51 months and in 14% of children aged <12 months.

In addition, *C difficile* coinfections were common in our study population. Twenty-five of the 35 patients with *C difficile* detection (71%) were also positive for an additional enteropathogen. Conversely, *C difficile* was detected in 17% of children with an EIA-confirmed rotavirus infection. These findings are in agreement with a recent study of US children with AGE, in which nearly 25% of those positive for *C difficile* had a virus detected concomitantly.[30] Also in that study, the children with a coinfection were younger and had a higher bacterial burden of *C difficile* than those with *C difficile* alone, yet they were otherwise clinically indistinguishable. Similarly, in our study, children with multiple pathogens detected were no more likely than other children with AGE to have fever, be hospitalized, or experience vomiting. In addition, we found no significant differences in clinical characteristics between children with *C difficile* detection alone and those with *C difficile* detected with another enteropathogen, although our analysis was limited by a small

sample size (Table IV; available at www.jpeds.com). Unfortunately, antibiotic use and hospitalization history, which are relevant to CDI, were not collected in the initial NVSN study and thus were unknown in these patients. Interestingly, we observed high rates of vomiting in our cohort of children with *C difficile* detection alone (10 of 11; 91%), higher than previously reported rates.[19,31] Reasons for this pattern are unclear, but the results may suggest a non–*C difficile* etiology of AGE in these patients or a higher rate of vomiting in younger children with CDI.

A recent switch from toxin-based *C difficile* testing to molecular tests has also raised concerns, as hospitals have reported a 50%-100% increase in CDI rates with this transition.[32,33] A recent study in adults found that virtually all CDI-related complications occurred in patients with a positive toxin immunoassay.[9] In that study, patients with a positive molecular test result and a negative toxin immunoassay were clinically comparable with those without CDI. The authors concluded that the exclusive reliance on molecular tests for CDI diagnosis resulted in overdiagnosis, overtreatment, and associated increased healthcare costs. In the present study, we found high *C difficile* detection rates in young children and those with other infectious detections. This information, coupled with a lack of clinical differences, suggests that at least a proportion of our *C difficile* detections in children with AGE reflect mere colonization. With the ever-expanding commercialization of multiplex nucleic acid–based testing for gastrointestinal infections, ongoing refinements to the clinical interpretation will be required, particularly in the setting of CDI.

Of note, for the majority of patients with AGE, an etiologic diagnosis will not affect management. Indeed, rotavirus laboratory confirmation and coding are uncommon in clinical settings.[34] In children, etiologic testing is often reserved for those who are more severely ill or have additional comorbidities. In these situations, testing for a constellation of possible causes may help expedite definitive management compared with traditional laboratory methods or single-target molecular assays.

Our study has several limitations. Although we comprehensively assessed the most common causes of AGE in our cohort of children from Davidson County, Tennessee, there is likely to be regional variation in the spectrum of pathogens, and thus our study may lack external validity. The duration of enrollment of healthy controls was also limited and might not reflect seasonal trends. In addition, although our cohort of healthy controls did not exhibit any signs or symptoms of AGE within 14 days before enrollment, it is possible that the few instances of pathogen detection were related to persistent shedding from a preceding AGE episode. The small number of control patients in our study, along with the lack of clinical information preceding the 14-day symptom-free window, limit our ability to draw additional conclusions about subclinical pathogen shedding and relevance of positive test results in asymptomatic patients. This same principle applies to the AGE population, in which the high sensitivity of nucleic acid amplification testing might detect genetic material from pathogens that are nonviable or associated with a previous, clinically resolved infection. Of note, no etiologic agent was identified in 30% of our patients with AGE. These children may have been ill owing to noninfectious vomiting and/or diarrhea, or the AGE symptoms may have been caused by novel enteric pathogens not included in our analysis.

Finally, we used the Kruskal-Wallis test for statistical analyses of continuous variables among infection groups. If there was a statistically significant finding, then additional pairwise comparisons were performed using the Wilcoxon rank-sum test with Bonferroni correction. This is a notably conservative statistical method, which may carry an increased risk of a type II error and failure to reject a false null hypothesis.

Despite the substantial burden of AGE in children, the full spectrum of causative pathogens and their clinical implications have not been fully defined. Using GPP and a duplex RT-rtPCR assay for sapovirus and astrovirus, we identified an enteropathogen in 70% of children with AGE, compared with only 11% of asymptomatic controls. Detection of enteropathogens in a substantially larger proportion of patients with AGE compared with asymptomatic controls supports the general clinical validity of multitarget molecular assays for the etiologic diagnosis of pediatric AGE; however, these results also demonstrate that the presence of microbial nucleic acid is not a perfect diagnostic biomarker, because healthy children may also test positive for known pathogens. Although PCR-based analysis of stool specimens using a multiplex approach offers extended insights into current potential causes of diarrheal illness in young children. Challenges in data interpretation remain, particularly those involving coinfections and *C difficile*.

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Glossary

AGE	Acute gastroenteritis
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* infection
EIA	Enzyme immunoassay
GII	Genogroup II
GPP	Gastrointestinal Pathogen Panel
MCJCHV	Monroe Carell Jr Children's Hospital at Vanderbilt
NVSN	New Vaccine Surveillance Network

RT-rtPCR Reverse transcription real-time polymerase chain reaction

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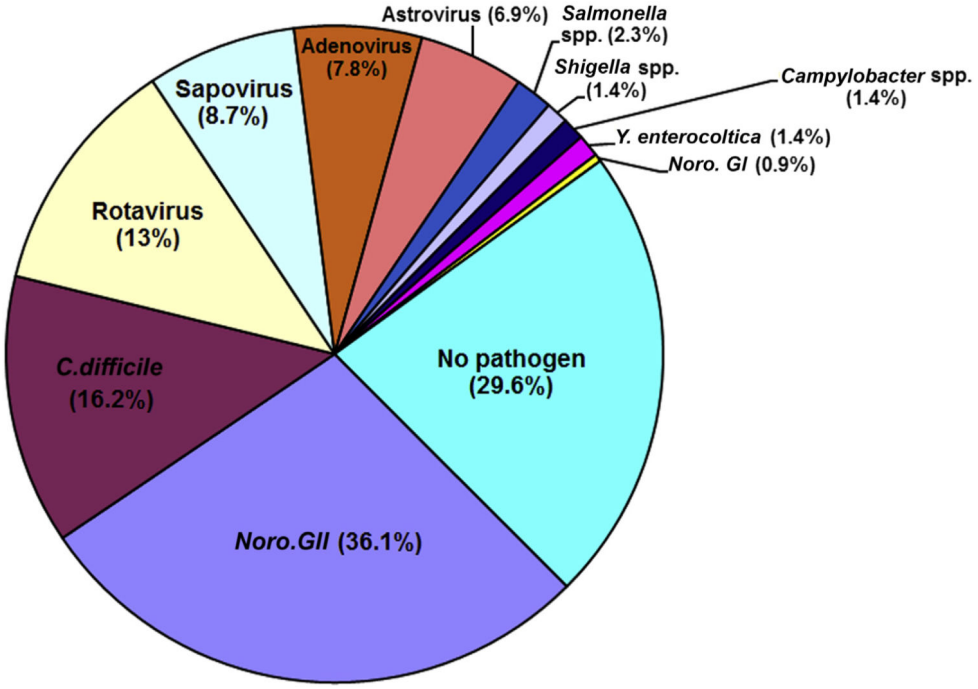

Figure 1.
Distribution of detected pathogens in the patients with AGE (n = 216).

	Noro. GII	C difficile	Rotavirus	Sapovirus	Astrovirus
C difficile	16				
Rotavirus	4	1			
Sapovirus	4	4	3		
Adenovirus	2	3		2	
Astrovirus	2	1	4	2	
Salmonella spp.	1	1		1	1
Shigella spp.	1				1
Campylobacter spp.	2		1		1
Y enterocolitica	1	1			
Noro. GI	1	1			

Dual infection summary includes five co-infections with three pathogens each:

C difficile + norovirus GI + norovirus GII
C difficile + adenovirus + norovirus GII
C difficile + *Salmonella spp.* + sapovirus
Campylobacter spp. + rotavirus + astrovirus
norovirus GII + rotavirus + astrovirus
rotavirus + astrovirus + sapovirus
rotavirus + norovirus GII + sapovirus

Figure 2.
Frequency of coinfections in the patients with AGE.

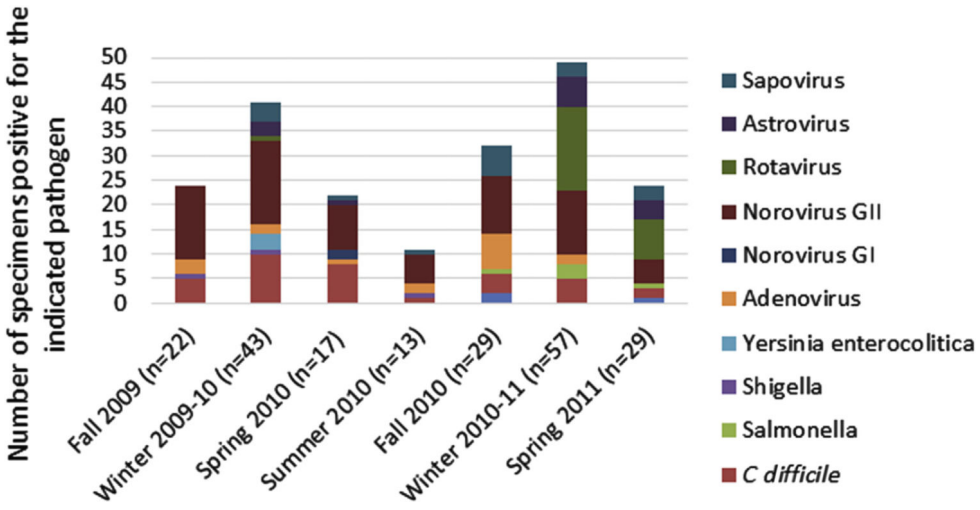

Figure 3.

Seasonal variation of pathogen detection in the patients with AGE (n = 210). Owing to the low numbers of specimens tested, specimens from spring 2008, spring 2009, and summer 2009 are not shown for clarity.

Table I

Gastrointestinal pathogen targets

Bacteria	Viruses	Parasites
Campylobacter spp	Adenovirus serotypes 40 and 41	*Cryptosporidium* spp
C difficile (toxins A and B)	Astrovirus*	*Entamoeba histolytica*
Enterotoxigenic *E coli* (heat-labile and heat-stable toxins)	Norovirus GI	*Giardia* spp
E coli O157	Norovirus GII	
Salmonella spp	Rotavirus group A	
Shiga toxin–producing *E coli* (Shiga toxins 1 and 2)	Sapovirus*	
Shigella spp		
Vibrio cholera		
Yersinia enterocolitica		

GI, Genogroup I.

*Targets were detected using the Analyte-Specific Reagent GI Panel with the exception of astrovirus and sapovirus, for which a duplex RT-rtPCR assay was used.[13]

Table II

Determination of positive specimens

Assay	Pathogen	Determination of specimen positivity
GPP* performed at Vanderbilt with or without GPP and/or single-plex PCR performed at the Luminex Molecular Diagnostics Laboratory	*Campylobacter* spp	Screen-positive at Vanderbilt using GPP and subsequently confirmed at the Luminex Molecular Diagnostics Laboratory using single-plex and/or multiplex PCR with primers specific for pathogenic *Campylobacter* species (*jejuni*, *coli*, and *lari*)*
	C difficile	Codetection of toxin A and toxin B or reproducible detection of either toxin A or toxin B
	Enterotoxigenic *E coli* (heat-labile and heat-stable toxins)	Median fluorescence intensity ≥2 times the target-specific positivity threshold
	E coli O157	
	Salmonella spp	
	Shiga toxin-producing *E coli* (Shiga toxins 1 and 2)	
	Vibrio cholerae	
	Shigella spp	
	Yersinia enterocolitica	
	Adenovirus serotypes 40 and 41	
	Norovirus GI	
	Norovirus GII	
	Rotavirus group A	
	Cryptosporidium spp	
	Entamoeba histolytica	
	Giardia lamblia	
Duplex RT-rtPCR assay performed at Vanderbilt	Astrovirus	Target-specific Ct value ≤35
	Sapovirus	

GI, Genogroup I.

*It was discovered that *Campylobacter* primer sets in the GPP cross-reacted with nonpathogenic *Campylobacter* species. Therefore, *Campylobacter*-positive specimens were sent to the Luminex laboratory for confirmatory PCR testing using targets specific for pathogenic species. The final analysis of *Campylobacter* infections reflects only those specimens containing pathogenic species. One positive specimen was unavailable for confirmatory PCR and treated as negative.

Table III

Demographic and clinical characteristics of patients with AGE by pathogen

Characteristics	Viral pathogen detected (n = 90)	Bacterial pathogen detected (n = 13)	Coinfection detected (n = 49)	No pathogen detected (n = 64)	*P* value
Age, mo, median (IQR)	19.5 (9-30)	10 (4-16)	12 (8-22)	14.5 (3-26)	.04 *
Male sex, n (%)	51 (57)	6 (46)	24 (49)	36 (56)	.75 †
Race, n (%)					.09 †
White	32 (36)	3 (23)	15 (31)	16 (25)	
Black	26 (29)	6 (46)	26 (53)	27 (42)	
Other	32 (36)	4 (31)	8 (16)	21 (33)	
Breastfeeding, n (%)					
Current	9 (10)	1 (8)	1 (2)	12 (19)	.14 †
Ever	58 (64)	8 (62)	35 (71)	49 (77)	.38 †
Daycare attendance, n (%)	27 (30)	3 (23)	20 (41)	16 (25)	.29 †
Hospitalization, n (%)	26 (29)	6 (46)	14 (29)	24 (38)	.44 †
Length of hospitalization, d, median (IQR)	1 (1-2); n = 26	0.5 (0-1); n = 6	1 (1-2); n = 14	2 (1-3); n = 24	.002 *
Fever during illness, n (%)	50 (56)	11 (85)	29 (59)	43 (67)	.38 †
Highest temperature, °F, median (IQR)	102 (101-103); n = 39	101 (100-102); n = 7	102 (101-103); n = 25	102 (101-103); n = 39	.26 *
Vomiting during illness, n (%)	82 (91)	11 (85)	41 (84)	53 (83)	.43 †
Maximum number of episodes of vomiting in 24 h, median (IQR)	5 (3-8); n = 80	3.5 (2-6); n = 8	4 (3-6.5); n = 40	4 (2-6); n = 51	.54 *
Maximum number of diarrheal episodes in 24 h, median (IQR)	6 (4-8); n = 74	6.5 (4-15); n = 10	6.5 (3.5-10); n = 40	3.5 (2-7); n = 46	.015 *
Rotavirus vaccine status, n (%)					.08 †
Yes	64 (71)	9 (69)	40 (82)	36 (56)	
No	20 (22)	4 (31)	8 (16)	25 (39)	
Unknown	6 (7)	0 (0)	1 (2)	3 (5)	

* Kruskal-Wallis test.

† Pearson test.

Table IV

C difficile detections

Variables	*C difficile* detection alone (n = 11)	*C difficile* coinfection (n = 24)	*P* value
Hospitalization, n (%)	5 (45)	7 (29)	.35 *
Length of hospitalization, d, median (IQR)	1 (1-2); n = 7	1 (0-1); n = 5	.37 †
Fever during illness, n (%)	8 (73)	13 (54)	.30 *
Highest temperature, °F, median (IQR)	101.5 (101-102); n = 6	102 (100-102); n = 10	.69 †
Vomiting during illness, n (%)	10 (91)	21 (88)	.77 *
Maximum number of episodes of vomiting in 24 hours, median (IQR)	3 (2-5.5); n = 8	4 (3-7); n = 20	.24 †
Maximum number of diarrheal episodes in 24 hours, median (IQR)	6 (2-15); n = 7	5 (3-8.5); n = 20	.76 †

* Pearson test.

† *t* test.