Preplanned Studies

Epidemiological Insights into Foodborne Pathogens Through qPCR Exploration of Prevalence — Beijing Municipality, China, January 2022–April 2023

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Summary

What is already known on this topic?

Foodborne diseases present a substantial global health risk. Traditional diagnostic methods have constraints, but advancements in molecular techniques, like quantitative polymerase chain reaction (qPCR), provide a hopeful solution.

What is added by this report?

We examined 1,011 stool samples from individuals suspected of foodborne illnesses. Our analysis indicated a significant presence of *Clostridium perfringens*, *Salmonella enterica*, enterotoxigenic *Escherichia coli* (ETEC), and adenovirus. Notably, co-infections were identified in 71.22% of the samples.

What are the implications for public health practice?

The data emphasize a notable prevalence of co-infections, highlighting the complexity of foodborne illnesses. This study underscores the significance of utilizing contemporary diagnostic methods in densely populated urban areas such as Beijing Municipality.

Foodborne illnesses pose a persistent threat to global health, commonly manifesting as acute gastroenteritis due to infections spread via contaminated food or water (*1*). With the increasing globalization and complexity of our food networks, there is an imperative need for rapid and precise pathogen detection methods (*2*–*3*). This is especially true in Beijing Municipality, where the distinctive demography and environmental factors underscore the importance of employing advanced diagnostic techniques (*4*–*6*). In our investigation, we collected 1,011 stool specimens from patients with presumed foodborne illnesses at 28 health facilities within the city. Utilizing quantitative polymerase chain reaction (qPCR), we identified and enumerated 35 different foodborne pathogens. The findings revealed a significant incidence of *Clostridium perfringens*, *Salmonella enterica*, enterotoxigenic *Escherichia coli* (ETEC), and adenovirus. Remarkably, 71.22% of the samples exhibited multiple concurrent infections. It is crucial for public health officials to consider culture-independent diagnostic tests (CIDTs) for disease identification and the prevalence of co-infections, which will notably improve the monitoring, prevention, and management of foodborne diseases in metropolitan areas. From January 2022 to April 2023, we amassed a total of 1,011 stool specimens from patients diagnosed with foodborne diseases in 28 different hospitals across Beijing. These patients were identified as having a foodborne disease based on symptoms that included recurring watery stool, mucus-laden or bloody stool, or vomiting, occurring three or more times within a 24-hour span, and reported as potentially linked to food consumption. We categorized patients by age: children (≤5 years old), adolescents (6–17 years old), adults (18–64 years old), and seniors (≥65 years old).

Each patient provided a fresh fecal sample weighing 5 mg, preserved in Cary-Blair transport medium CM0935 (Oxoid, Basingstoke, Hampshire, United Kingdom) at 4 °C. The samples were then transported to a designated laboratory within 24 hours for nucleic acid extraction. Nucleic acids were extracted from the fecal samples at the laboratory using a rapid nucleic acid extraction instrument NE-02-K-96 (Guangzhou Baybio Bio-tech Co., Ltd. Guangzhou, Guangdong, China) and a commercially available extraction kit STNM-48-K (Guangzhou Baybio Bio-tech Co., Ltd. Guangzhou, Guangdong, China). Detection of foodborne pathogens was carried out using single qPCR, with specific primers and probes obtained from literature or custom-designed for this study. Details of the pathogens detected, along with their primer and probe sequences, qPCR cycling conditions, and templates are listed in Supplementary Table S1 (available at https://weekly.chinacdc.cn/).

Statistical analysis was conducted using SPSS

software (version 20.0; IBM SPSS, Chicago, IL, USA). The frequencies of different pathogens among patients in various age groups were compared using χ^2 and Fisher's exact tests for dichotomous variables. A *P*-value of <0.05 was considered statistically significant.

A total of 1,011 eligible stool samples were collected, and nucleic acid extraction was successful for all samples. The detection rate for foodborne pathogens overall was 92.48% (935/1,011).

Clostridium perfringens had the highest positivity rate at 52.03% among the samples analyzed (Figure 1A), followed by *Salmonella enterica*, ETEC, and adenovirus with rates of 20.67%, 20.97%, and 19.88%, respectively. Enteropathogenic *Escherichia coli* (EPEC) and enteroaggregative *Escherichia coli* (EAEC) were also prevalent, detected in 19.49% and 15.13% of the samples. Pathogens found in 5% to 15% of the samples included *Staphylococcus aureus* (*S. aureus*), *Campylobacter jejuni*, *Bacteroides fragilis*, *Clostridium difficile*, rotavirus, *Vibrio parahaemolyticus*, *Shigella*/Enteroinvasive *Escherichia coli.* (EIEC), *Cronobacter* spp., *Aeromonas* spp., norovirus, and *Vibrio cholerae*. Co-infections were present in 71.22% (720/1,011) of the samples (Table 1). Most infections involved one, two, or three pathogens, with rare cases having up to nine pathogens detected. Some cases of co-infections involved seven to nine pathogens, as detailed in Table 1 along with the onset time of these cases. Pathogen combinations for all samples are presented in Table 1. Pathogens were undetectable in 7.52% of the samples.

In spring, the prevalence of *S. aureus* was relatively

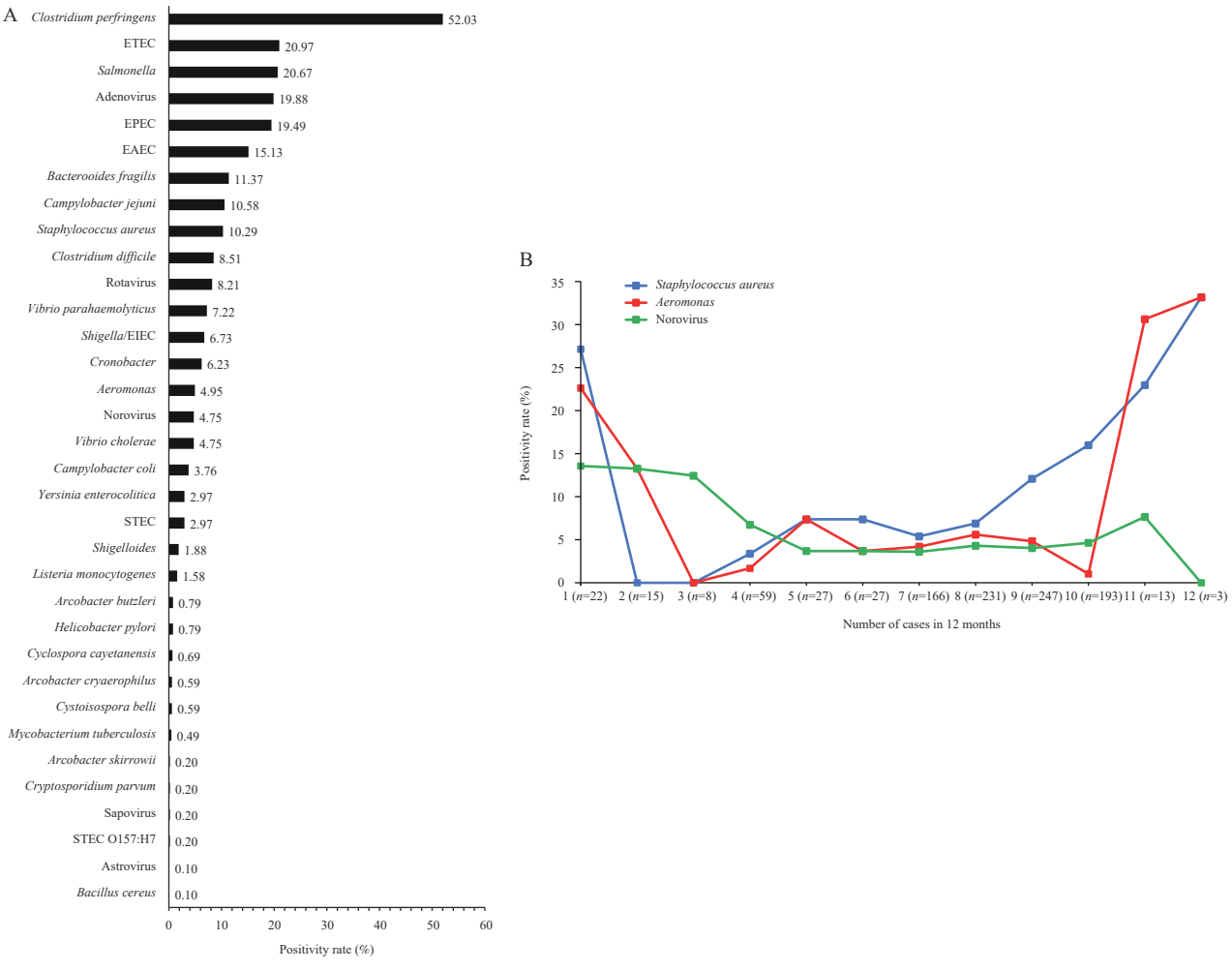

FIGURE 1. Prevalence of 35 pathogens in patients with acute diarrhea from Beijing. (A) Frequency distribution of 35 pathogens detected among 1,011 patients with acute diarrhea; (B) Seasonal distribution of *Staphylococcus aureus*, *Aeromonas* spp., and norovirus in 1,011 patients with acute diarrhea.
Abbreviation: ETEC=enterotoxigenic *Escherichia coli*; EPEC=enteropathogenic *Escherichia coli*; EAEC=enteroaggregative *Escherichia coli*; STEC=Shiga toxin-producing *Escherichia coli*; EIEC=Enteroinvasive *Escherichia coli.*

TABLE 1. Results of 1,011 stool samples from patients with foodborne diseases showing co-infections and infection patterns with 7–9 pathogens based on quantitative PCR experiments.

Pattern of infections	Time of onset	Cases (%)
Nonuple infections		2 (0.20)
Cronobacter spp. + *Clostridium perfringens* + STEC O157:H7 + STEC + *Clostridium difficile* + *Salmonella enterica* + EAEC + ETEC + adenovirus	Aug-22	
Clostridium perfringens + rotavirus + *Salmonella enterica* + EPEC + *Cystoisospora belli* + *Vibrio parahaemolyticus* + *Yersinia enterocolitica* + ETEC + adenovirus	Aug-22	
Octuple infections		4 (0.40)
Cronobacter spp. + *Staphylococcus aureus* + *Clostridium perfringens* + sapovirus + *Bacteroides fragilis* + *Campylobacter coli* + *Yersinia enterocolitica* + ETEC	Sep-22	
Staphylococcus aureus + *Clostridium perfringens* + STEC O157:H7 + STEC + *Campylobacter jejuni* + *Vibrio parahaemolyticus* + *Yersinia enterocolitica* + adenovirus	Sep-22	
Clostridium perfringens + rotavirus + *Salmonella enterica* + *Vibrio cholerae* + EAEC + *Campylobacter coli* + ETEC + norovirus	Aug-22	
Clostridium perfringens + *Clostridium difficile* + *Salmonella enterica* + *Campylobacter jejuni* + *Vibrio parahaemolyticus* + *Yersinia enterocolitica* + ETEC + adenovirus	Oct-22	
Septuple infections		8 (0.79)
Cronobacter spp. + *Clostridium perfringens* + *Salmonella enterica* + EPEC + *Plesiomonas shigelloides* + *Yersinia enterocolitica* + ETEC	Sep-22	
Cronobacter spp. + *Clostridium perfringens* + *Campylobacter coli* + *Cystoisospora belli* + *Vibrio parahaemolyticus* + ETEC + adenovirus	Aug-22	
Staphylococcus aureus + *Clostridium perfringens* + *Aeromonas* spp. + *Clostridium difficile* + *Salmonella enterica* + EAEC + *Cyclospora cayetanensis*	May-22	
Staphylococcus aureus + *Clostridium perfringens* + EAEC + *Campylobacter jejuni* + *Vibrio parahaemolyticus* + ETEC + adenovirus	Oct-22	
Clostridium perfringens + rotavirus + *Salmonella enterica* + *Vibrio cholerae* + EPEC + ETEC + adenovirus	Aug-22	
Clostridium perfringens + *Aeromonas* spp. + *Clostridium difficile* + EAEC + EPEC + *Campylobacter jejuni* + ETEC	Oct-22	
Clostridium perfringens + *Shigella*/EIEC + EAEC + EPEC + *Arcobacter skirrowii* + *Arcobacter cryaerophilus* + adenovirus	Aug-22	
Clostridium perfringens + EAEC + *Campylobacter jejuni* + *Vibrio parahaemolyticus* + ETEC + adenovirus + norovirus	Oct-22	
Sextuple infections		24 (2.37)
Quintuple infections		64 (6.33)
Quadruple infections		147 (14.54)
Triple infections		211 (20.87)
Duple infections		260 (25.72)
Single infection		215 (21.27)
Negative		76 (7.52)
Total		1,011

Abbreviation: PCR=polymerase chain reaction; ETEC=enterotoxigenic *Escherichia coli*; EPEC=enteropathogenic *Escherichia coli*; EAEC=enteroaggregative *Escherichia coli*; STEC=Shiga toxin-producing *Escherichia coli*; EIEC=Enteroinvasive *Escherichia coli*.

low, increasing from summer onwards to peak at 33.33% in December (Figure 1B). *Aeromonas* spp. exhibited a higher positive rate during winter, whereas norovirus showed increased rates in winter and spring, except for December due to limited samples. Seasonal variations were not observed in other pathogens.

An analysis of pathogen positivity rates across different age groups revealed significant differences (Figure 2). Notably, children showed a higher positivity rate for adenovirus compared to adolescents and the elderly. EPEC presented elevated positivity rates in both children and adults when contrasted with elderly populations. *S. aureus* was more frequently detected in children and adolescents rather than in adult and elderly groups. *C. difficile* displayed increased positivity in both children and elderly individuals relative to adults. In contrast, *V. cholerae* was less commonly identified in children than in adults, and *C. coli* was found to have lower positivity rates in children as opposed to the other three age cohorts.

DISCUSSION

In this study, we performed multipathogen testing on stool specimens from 1,011 individuals presenting

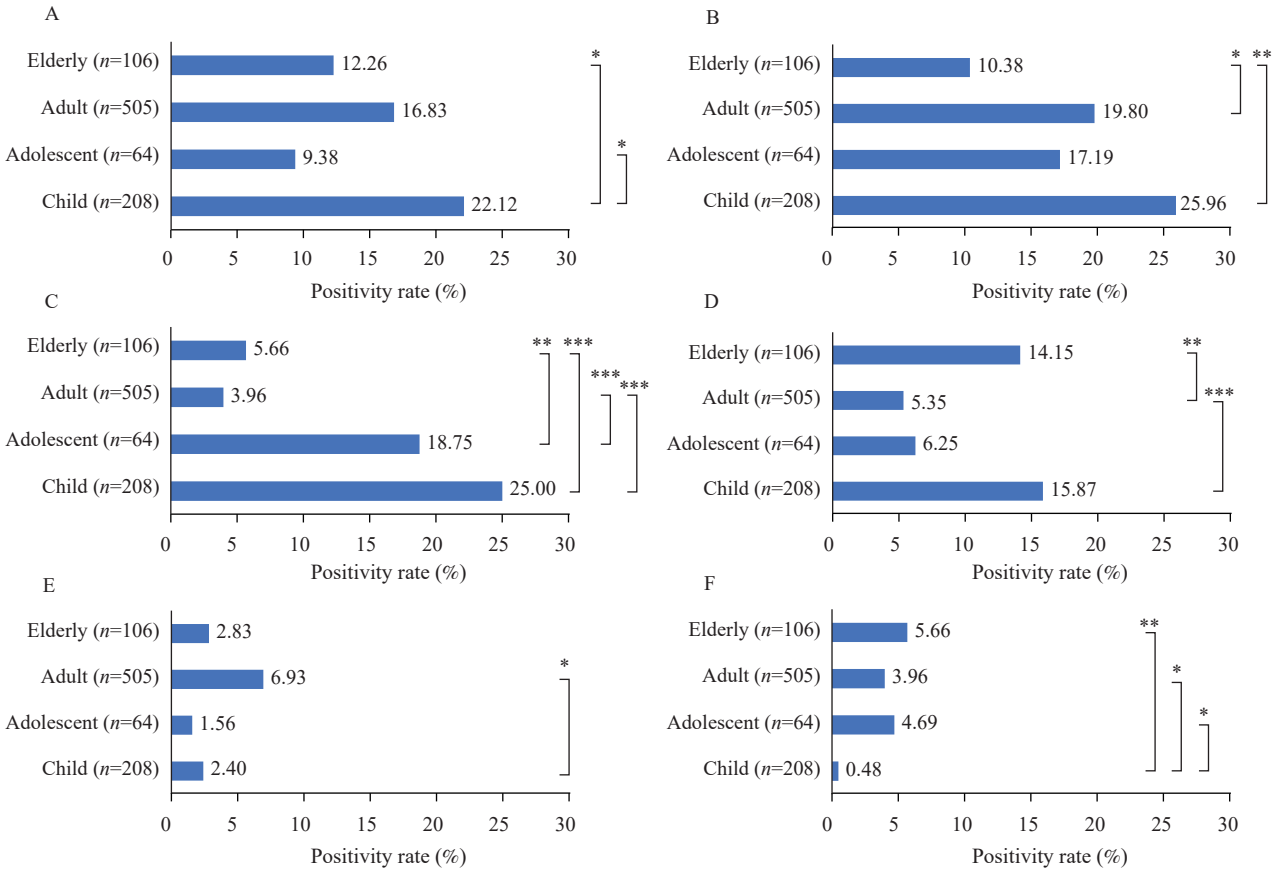

FIGURE 2. Isolation rates (%) of six pathogens across different age groups: (A) adenovirus, (B) ETEC, (C) *Staphylococcus aureus*, (D) *Clostridium difficile*, (E) *Vibrio cholerae*, (F) *Campylobacter coli*.
Note: Because age data was missing for some patients during data collection, only 883 samples were included in this analysis.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

with foodborne illness in Beijing to characterize the prevalence and diversity of pathogens implicated. Our analysis revealed a notable incidence of coinfections, which underscores the specific challenges and food safety vulnerabilities faced by the city. We observed a considerably high positivity rate for *C. perfringens*. However, this finding warrants a careful interpretation. Although traditionally associated with the consumption of improperly cooked or stored meats, *C. perfringens* has been found in 21.2%–36.0% of healthy individuals (*7*). Therefore, while the detection of *C. perfringens* is of interest, it does not invariably suggest a pathogenic role in each instance. This finding highlights the critical need to differentiate between true pathogens and commensal organisms within the gastrointestinal tract, offering insights for future efforts to identify bona fide pathogens.

The significant positivity rates for *Salmonella enterica*, ETEC, and adenovirus align with findings from other surveillance studies (*8*), indicating broad contamination sources, likely spanning from water sources to diverse food items. Elevated adenovirus detection in children underscores their susceptibility, highlighting the need for precise identification of adenovirus serotypes and tailored preventive measures in environments where children are commonly present (*9*).

The variations in seasonal patterns, including the increase in *S. aureus* rates from summer to December and the elevated rates of *Aeromonas* spp. and norovirus in winter and spring, highlight potential environmental triggers or behavioral patterns that could impact these trends.

Differences in pathogen prevalence across age groups are crucial for understanding disease prevention. Higher adenovirus rates in children may indicate age-

specific exposure or susceptibility. A study of 1,715 children demonstrated a high adenovirus positivity rate (*10*). Variances in *C. difficile* and other pathogen positivity rates by age imply age-related vulnerabilities or exposure routes.

The significant prevalence of coinfections raises interesting inquiries. When various pathogens are present in one host, identifying the main contributing pathogen to the clinical symptoms is difficult. It is crucial to investigate whether one pathogen is predominantly responsible for the symptomatic manifestation or if the combined effects of multiple pathogens worsen the severity of the disease. Unraveling this intricate interaction is a key focus for future research endeavors.

The study demonstrates the diverse diagnostic capabilities of qPCR-based molecular technology. It is suggested to integrate qPCR into pathogen surveillance for foodborne diseases and utilize it alongside epidemiological and clinical data to effectively inform public health interventions and policy modifications concerning foodborne disease surveillance.

This study is subject to some limitations. The analysis was solely reliant on CIDTs, and over half of the collected samples originated from adult participants, resulting in a lower representation of other age groups. Additionally, the sample collection intervals were not consistently distributed.

Despite these constraints, the sample sizes were adequate to identify trend patterns. To the best of our understanding, this is the most extensive survey of its kind conducted in China to date. We plan to extend our sample collection efforts and conduct concordance assessments between CIDT results and traditional culture methods. Furthermore, we will investigate the prevalence of various pathogenic sequences, including *Clostridium perfringens*, using metagenomic sequencing. Our future work will also involve contrasting the gut microbiota community structures of individuals with foodborne illnesses and those in good health, with the aim of informing more sophisticated strategies for the prevention and management of foodborne diseases.

Conflicts of interest: No reported conflicts.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Primer and probe sequences for quantitative PCR in this study.

Pathogen	Gene	Strand	Sequence	Reference	genbank code	product size (bp)
Cronobacter spp.	ompA	Forward	TGGTACGCAGGTGGCAAA	(*1*)	KU354278.1	68
		Reverse	GGGCCGTCGTTAGGAATAAA			
		Probe	TTGGTCCCAGTTCCACGATACCGG			
Staphylococcus aureus	nuc	Forward	GTTTTTCTATTTCGCTACTAGTTGTTTAGTG	(*1*)	LS483319.1	134
		Reverse	CACTATATACTGTTGGATCTTCAGAACCA			
		Probe	TCAGCAAATGCATCACAAACAGATAATGGC			
Bacillus cereus	crs2	Forward	AGGATTCTATGACACRGCTACT	This study	AB248763.2	159
		Reverse	GATKACCTGTACTAYCAGAAGT			
		Probe	TGACTTAGATGCSGCGAAATACCTTGC			
Clostridium perfringens	plc	Forward	CTTTGCTGCATAATCYCAA	This study	MK180795.1	257
		Reverse	CTGCTAATGTTASTGCCGTT			
		Probe	TCATCCYAACTATGACTCMTGCTAGC			
E.coli O157	rfbE	Forward	CGCCAAYCAAGATCCTMA	This study	CP114132.1	68
		Reverse	GTCCACACGWTGCCAATG			
		Probe	CGGAAAAATATCAAAAGCACSCTATRGC			
Aeromonas spp.	Aerolysin	Forward	GCCTGTCTACCATKCAGAAC	This study	MT491733.1	215
		Reverse	GCTCTYGCGGCATTMG			
		Probe	ACCTGGCCAGAGTGCTGCGC			
Bacteroides fragilis	bft	Forward	GGGACAAGGATTCTACCAGCTTTATA	(*2*)	AB026626.1	126
		Reverse	ATTCGGCAATCTCATTCATCATT			
		Probe	CAATGGCGAATCCATCAG			
Campylobacter jejuni	gyrA	Forward	GGGTGCTGTTATAKGTCGT	This study	KP159411.1	267
		Reverse	AAGACATCAGGTTCRCTTTCT			
		Probe	CATGAGAAAGYTTACTCATTTTTGC			
Campylobacter coli	glyA	Forward	GCGAGTGMTTATGCTCGTA	This study	AF136494.1	99
		Reverse	TCCAGCAATGTGTKCAAYG			
		Probe	TAGAGRGATTGCGGATGAAGYTGGAGC			
Clostridium difficile	tcdB	Forward	GGTATTACCTAATGCTCCAAATAG	(*2*)	MN625141.1	87
		Reverse	TTTGTGCCATCATTTTCTAAGC			
		Probe	CCTGGTGTCCATCCTGTTTC			
Clostridium difficile	tcdA	Forward	CTTCAAGSAGAAATAGWGCAC	This study	KC292125.1	275
		Reverse	TAGCTGTAATGCTTCAGYGGTA			
		Probe	TGGATAGGTKGAGAAGTCAGTGAKATTGCTC			
Helicobacter pylori	ure	Forward	GTGCTAGATACCGYTAATGG	This study	OL906288.1	206
		Reverse	CTGTCAGCATSGCCATCA			
		Probe	TGCGGCTTATAAGRTGGCTCCRG			
Listeria monocytogenes	hly	Forward	CGCAATCAGTGAAGGGRA	This study	MG922920.1	187
		Reverse	GCCATATGCCACACTTGMGAT			
		Probe	AGCAGTTGCWAGCGCTTGGAGTG			

Continued

Pathogen	Gene	Strand	Sequence	Reference	genbank code	product size (bp)
Mycobacterium tuberculosis	orfB	Forward	GGGTAGCAGACCTCACCTATG	(2)	MH883892.1	74
		Reverse	AGCGTAGGCGTCGGTGA			
		Probe	TCGCCTACGTGGCCTTT			
Plesiomonas shigelloides	glmU	Forward	CGTGATTGATACGGSACGTAGTA	This study	CP076372.1	113
		Reverse	GGATCACCMAATTCAGAKCATC			
		Probe	TCGGTGCCGAAARTATCCATCTGATCTAC			
Salmonella enterica	ttrC	Forward	GGCTATTCTCKGCACCTT	This study	CP126323.1	77
		Reverse	TCCTGGTGAGTCCSTTCA			
		Probe	CGGCCTGTGGATAGCGCTACTGA			
Salmonella enterica	ompC	Forward	ACCGCTAACGCTCGCCTGTAT	(2)	CP055130.1	122
		Reverse	CGGGTTGCGTTATAGGTCTGA			
		Probe	AATACTGCGCTGCCAGAT			
Salmonella enterica	invA	Forward	CGATCAGTACCAGTCGTYTT	This study	CP121298.1	89
		Reverse	CAGGCTATCGCCAATAMCG			
		Probe	CTTGATTGAASCCGATGCYGGT			
Shigella/EIEC	ipaH	Forward	CCTTTTCCGCGTTCCTTGA	(2)	CP130064.1	64
		Reverse	CGGAATCCGGAGGTATTGC			
		Probe	CGCCTTTCCGATACCGTCTCTGCA			
Vibrio cholerae	hlyA	Forward	ATCGTCAGTTTGGAGCCAGT	(2)	MF100000.1	102
		Reverse	TCGATGCGTTAAACACGAAG			
		Probe	ACCGATGCGATTGCCCAA			
Vibrio parahaemolyticus	toxR	Forward	GCGGAGAGWCCAARCGAAGT	This study	MH047287.1	148
		Reverse	ACTCKGGAGATTTGGTTGAATC			
		Probe	TGGCGTGAGCAAGGTTWTGAGGTGGAT			
Yersinia enterocolitica	cadR	Forward	GCAGATAGCAGACMTGCATYCT	This study	LR134161.1	215
		Reverse	GGTATTSCTGCTGGTGAATCAA			
		Probe	AACACTAAGGTGAACGGGCTGACGCTA			
Arcobacter cryaerophilus	feoB	Forward	TGACATTATCCAWGCRGTTGTTG	This study	CP060692.1	208
		Reverse	GAGTGAAAATCCAGATGAACSAA			
		Probe	ACAAGRTATTTCCATCSTCCAGCTT			
Arcobacter skirrowii	nadB	Forward	TCCTTGTATCTCTCCACTRTCAG	This study	CP032099.1	199
		Reverse	CTCATCRCAACTAAWCTMTGAATC			
		Probe	CCAAATCSGCCACTTGCTAAAATTKTATTGTG			
Arcobacter butzleri	glyA	Forward	GCAATTGATAGAGCTYGTGAA	This study	AF136498.1	209
		Reverse	ACTCCATAATAGMATGCTTGRT			
		Probe	AGCCAMGCAAATGGAGCAGTWTATGCA			
STEC	sta	Forward	GCACAGGCAGGATTASAACA	This study	KY581592.1	227
		Reverse	AACAACATGWCGGGAGGTARCA			
		Probe	AGTTCACAGCAGTMAAATGTGYTGTTC			
STEC	stx1	Forward	CCAGGTACAACAKCGGTTAC	This study	OP785750.1	155
		Reverse	TGCGTCAGTGAGGWTYCA			
		Probe	TGTCTGGTGACASTAGCTATACCACKTTACA			

Continued

Pathogen	Gene	Strand	Sequence	Reference	genbank code	product size (bp)
		Forward	GCTGATGCYGACGATWCTGT			
EAEC	aggR	Reverse	GTGTWTCTGACSTTATCGGAA	This study	MT471349.1	173
		Probe	AGAGTCAATTTATATATCGGCTGTRAGCTTCT			
		Forward	GCGAATACTGGCSAGWCTA			
EPEC	eae	Reverse	GATTCGMCTGCAACTTATCG	This study	LC504610.1	227
		Probe	AGTAGCGTTAACGGCTATTTCCKC			
		Forward	TGACGCTTACCASGTYGGAT			
EPEC	bfpA	Reverse	TAACACCGTAGCCTTTCGCT	This study	AF304485.1	84
		Probe	AGCGGCATGTSTTAGTCTTRCAACCTTG			
		Forward	GGCAGAGGATKGTTMCAGAT			
ETEC	eltA	Reverse	AATCCAGGGTTCTTCTCTSCAA	This study	KF733767.1	70
		Probe	AGCAGGTTTCCCACCGGATCACCAA			
		Forward	CGGTACAAGCAGGATWACAAC			
ETEC	estA	Reverse	ACCTTTCSCTCAGGATGCTA	This study	AJ868113.1	161
		Probe	CAGCAGTAATTGCTACYATTCATGC			
		Forward	CCACATCGGTGTCTGTTATTAACC			
STEC	stx2	Reverse	GGTCAAAACGCGCCTGATAG	(2)	AP025741.1	93
		Probe	TTGCTGTGGATATACGAGG			
		Forward	CTCTCGCCCTGCGAAGTAA			
Adenovirus F	fiber	Reverse	GGCGTCTATTAAKAGRGAAAGT	This study	MK854763.1	137
		Probe	TACAACGCTSCCTTAAACGTAG			
		Forward	GTTGRCGGAGAGGGCTWCAA			
Adenovirus C	hexon	Reverse	GCATCTGWACCAAGAACMAKTCT	This study	MH322276.1	67
		Probe	CGTTGCCCMATGCAACAYGAC			
		Forward	TCAGATGATGATGATGTTGAGAAC			
Astrovirus	capsid	Reverse	CCAACAGGTCRTTGTAGACACT	This study	OQ633093.1	154
		Probe	AGGAATGTCAGTGGAKCGCGSCACAAG			
		Forward	CAGGCCRTGTTCCGCYSGAT			
Norovirus GI	capsid	Reverse	TCCTTAGACGCCATSATCAWTTAC	This study	AB058529.1	99
		Probe	TGTGGACAGGGSATCGYGATCT			
		Forward	AGGTTAATGCTWCTGAYCCTCTT			
Norovirus GI.1	capsid	Reverse	CTTGKGGAGCCTRCWCAAA	This study	KP753266.1	126
		Probe	CTCTTCAASAGCAGYTSCAACTG			
		Forward	CAGCTCMTGGYASTGCTT			
Norovirus GII	RdRp	Reverse	AACCTCAYCCACCTRAACAT	This study	MK280938.1	146
		Probe	TGAAGCCTCTSTTCACGRACCCT			
		Forward	CCCAGACAAKAGCCAWTGT			
Norovirus GII.4	RdRp	Reverse	TTGTGAATGAAGATRGCGYC	This study	MF158179.1	102
		Probe	AGATGGATGAGATTCWCAGAYCTGA			
		Forward	ACCATCTWCACRTRACCCTCTATGAG			
Rotavirus	NSP3	Reverse	GGTCACATAACGCCCCTATAGC	(2)	ON992748.1	87
		Probe	AGTTAAAAGCTAACACTGTCAAA			

Continued

Pathogen	Gene	Strand	Sequence	Reference	genbank code	product size (bp)
Sapovirus	RdRp	Forward	ATGCTTMACAWCATKGACCT	This study	MN245682.1	144
		Reverse	CTGTASCASCTATGAACCAAG			
		Probe	TGTGTTTGACACCGTRCGCCAAAT			
Cryptosporidium parvum	819-1080	Forward	CCAAGGYAGTSTAACACCAT	This study	XM_001388121.1	262
		Reverse	AGCATCATCTKATGAACTMCAAGT			
		Probe	CGATTGTTRACCTTCWTCCTGTTCACT			
Cyclospora cayetanensis	3859-4084	Forward	GCACARGATCGAGAWTCTAATG	This study	NW_020312297.1	226
		Reverse	GCAACAATCGAKTCCATAGTCAA			
		Probe	TGACGGCCTKTGATGCACCTWGCCG			
Cystoisospora belli	5.8S rRNA	Forward	CAGTSTCTCTGAAGTTTCWAGTTC	This study	MT835288.1	189
		Reverse	TTCGGGACACAACTCRACRCT			
		Probe	CTCACGSGCTTCTGGRGGTGTCTCT			
bacterial 16s	16S rRNA	Forward	GACGATCAGTAGCCGACTT	(*2*)	MT356186.1	199
		Reverse	GCTTCTTAGTCAAGTACCGTCA			
		Probe	AGAGAGTGATCGGCCACATTGGGA			
MS2	MS2g1	Forward	TGGCACTACCCCTCTCCGTATTCAC	(*2*)	LC710218.1	99
		Reverse	GTACGGGCGACCCCACGATGAC			
		Probe	CACATCGATAGATCAAGGTGCCTACAAGC			
PhHV	gB	Forward	GGGCGAATCACAGATTGAATC	(*2*)	Z68147.1	89
		Reverse	GCGGTTCCAAACGTACCAA			
		Probe	TATGTGTCCGCCACCATCT			

Abbreviation: PCR=polymerase chain reaction; ETEC=enterotoxigenic *Escherichia coli*; EPEC=enteropathogenic *Escherichia coli*; EAEC=enteroaggregative *Escherichia coli*; STEC=Shiga toxin-producing *Escherichia coli*; EIEC=Enteroinvasive *Escherichia coli*; MS2=bacteriophage MS2; PhHV=Phocine Herpesvirus.

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