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A DNA microarray for the versatile diagnosis of infectious diarrhea

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Several bacteria, viruses, and parasites cause diarrhea as coinfecting pathogens. We designed a DNA microarray comprising 60-bp probes spotted 194 times for the multiplex detection of 33 enteropathogenic bacteria and seven enteropathogenic viruses, and the archaeon *Methanobrevibacter smithii* was used as an internal positive control. Nine pathogen-free stool specimens were used as negative controls. One of these control specimens was further spiked with *Salmonella enterica* as a positive control. The microarray was then tested with 40 pathological stool specimens, comprising *S. enterica* (n = 30), *Campylobacter jejuni* (n = 4), pathogenic *Escherichia coli* (n = 2), and adenovirus (n = 4). *M. smithii* was detected in 47/49 (95.9%) specimens, no pathogen was detected in negative controls and *S. enterica* was identified in the *S. enterica*-spiked positive control. The overall specificity was 100% and the overall sensitivity was 97.5% because one *S. enterica* sample was missed by the microarray. The multiplexed detection of *C. jejuni* spiked into an adenovirus-positive stool sample gave positive results, with fluorescence values of 14.3 and 9.1, respectively. These data indicate that using the protocol developed in this article, the DNA array allows for the multiplexed detection of some enteropathogens in stool samples.

Key words: DNA microarray; diagnosis; infectious diarrhea.

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Infectious diarrhea is estimated to be the fifth leading cause of death worldwide, with an estimated 2.16 million deaths a year, including 1.5 million pediatric deaths (http://who.int/en/). In France, diarrhea is estimated to generate approximately three million yearly visits to a general practitioner (1). Pathogens known to be responsible for diarrhea include the bacteria *Campylobacter* spp. *Salmonella* spp., *Clostridium difficile,* pathogenic *Escherichia coli*, *Shigella* spp., and *Yersinia enterocolitica* (2) (http://www.ecdc.europa.eu/en/Pages/home.aspx). Viruses, including noroviruses, rotaviruses, toroviruses, coronaviruses, astroviruses, enteroviruses, and adenoviruses, reportedly cause 50% of cases of diarrhea (3). In particular, noroviruses are now the leading cause of diarrhea and enteritis outbreaks worldwide (4, 5).

Routinely, human enteropathogenic bacteria and viruses are searched for separately in different clinical laboratories in hospitals, but coinfections have been reported, particularly in developing countries (6–9). Therefore, a multiplex detection approach is warranted to speed diagnosis for the proper treatment and isolation of contagious patients. In addition, such an approach would allow for the detection of clusters and epidemics. A DNA microarray is such a technology for the rapid multiplexed detection of microorganisms in clinical specimens (10–14). Accordingly, DNA microarrays have been used to investigate stool microbiota

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(15–19). However, the use of DNA microarrays for the identification of enteropathogenic bacteria in human diarrheal stool specimens has been rarely reported (10, 12, 13, 20–23). These microarrays detected a few bacterial pathogens, and few DNA microarrays allowed for the multiplexed detection of pathogens (10–14).

We therefore customized a DNA microarray for the multiplex detection of 40 bacterial and viral enteropathogens and the archaeon *Methanobrevibacter smithii* as an internal control, which should be positive in all cases (24).

MATERIALS AND METHODS

Stool specimens

Nine pathogen-free stool specimens with normal consistency collected from healthy individuals were used as negative controls in all experiments. The control of carriage *Staphylococcus aureus* in stool is mandatory for some workers in hospital under French law. These stools were used as 'healthy individuals' without diarrhea. One of these control specimens was spiked with 10^8 colony-forming units (CFU)/mL (final concentration) *Salmonella enterica* CIP 60.62 serotype Typhimurium (Collection de l'Institut Pasteur, Paris, France) in phosphate-buffered saline (PBS) and used as a positive control. Human diarrheal stool specimens routinely submitted to the Méditerranée Infection clinical microbiology laboratory, Marseille, containing *S. enterica* (n = 30), enteropathogenic *Escherichia coli* (EPEC) (n = 1), enterohemorrhagic *E. coli* (EHEC) (n = 1), *Campylobacter jejuni* (n = 4), and adenoviruses (n = 4) were collected. Bacteria were routinely detected by culture methods, as previously described (2). Caliciviruses and enteroviruses were routinely detected by a specific real-time PCR method using previously described primers (25, 26). Rotaviruses were detected by an immunochromatographic assay (Standard Diagnostics, Gurgaon Haryana, India). All of the viruses were further detected by electron microscopy observation. Among these 40 diarrheal stools, no stool specimen was co-infected. No written consent was needed for this work in accordance with the 'LOI n° 2004–800 relative à la bioéthique' published in the 'Journal Officiel de la République Française' on August 6, 2004 because no additional sample was taken for the study. According to this law, patients were informed that stool specimens could be used for anonymized studies. This study was approved by the local ethics committee of the Institut Fédératif de Recherche 48, Faculty of Medicine, Marseille, France, reference number 08-002.

Microarray design

The archaeon *M. smithii* was used as an internal positive control, as we previously showed that it was detected in 95.7% of human stool specimens (24). To choose the 40 pathogens present on our DNA microarray, we based on a recent review of infectious diarrhea (2). DNA probes were designed on the basis of the 16S rRNA gene sequence for 15 bacterial enteropathogens and specific gene sequences for an additional 13 bacterial enteropathogens as well as for viruses spotted on our microarray (Tables 1 and 2). Among these pathogens, we designed one specific probe for the detection of *Grimontia hollisae* that is responsible of human diarrhea for people who consumed raw shellfish, especially oysters or more rarely raw or undercooked fish (27), and *Klebsiella oxytoca* that is responsible of antibiotic-associated hemorrhagic colitis (AAHC) especially in children (28). There are unpublished internal evidences of the association between *Planctomycetes* and the intestinal microbiota (Drancourt M, 2012, unpublished data), that is why we designed specific probes for the detection of *Gemmata obscuriglobus, Pirellula staleyi, Planctomyces brasiliensis/maris, Planctomyces limnophilus*, and *Rhodopirellula baltica*. In particular, five probes were spotted for the detection of pathogenic *E. coli*: for enterohemorrhagic *E. coli* (EHEC), we spotted *eae* and *stx1* gene probes (29); for enteroinvasive *E. coli* (EIEC), we spotted *ipaB* and *ipaD* gene probes (30); for enteropathogenic *E. coli* (EPEC), we spotted the *eae* gene probe (31); and for Shiga toxin-producing *E. coli* (STEC), we spotted *stx1* and *stx2* genes probes (32). The DNA microarray (Agilent Technologies, Massy, France) comprised eight hybridization arrays containing 15 744 features, each consisting of two interlaced rectangular grids of 96 rows at 0.073323–millimeter spacing by 82 columns at 0.127–millimeter spacing. Each 60-mer probe had an approximately 80 °C hybridization temperature. Each probe was spotted 194 times on each hybridization array.

DNA extraction

Diarrheal stools were lyophilized before DNA extraction. Briefly, stool specimens were freeze-dried for 24 h in 1-mL glass containers (Dominique Dustcher, Brumath, France) in the same lyophilizer with the negative control stool specimen. After lyophilization, stool specimens were regenerated in 250 µL PBS, resulting in a four-fold concentration of the diarrheal stool specimens. Lyophilized specimens were then manipulated in parallel with non-diarrheal stool specimens, which were not lyophilized. Instead, one gram of non-diarrheal stool specimen was diluted into 5 mL PBS and vortexed with 3-mm glass beads (Dominique Dustcher) for 30 s. In total, 250 µL of

Table 1. Probe sequences targeting bacteria

	Bacteria	Sequences	TM (°C)	Length (bp)
Intestinal pathogens	*Aeromonas caviae*	TTGTATGGAT ACCTTTTTAG AACAATTAAA GTGTGGATTC GATCGCATTC GTTGATTTCT	80.4	60
	Arcobacter butzleri	ATATGAACTT CTGCATTCAC TGTTCCCATT T CTATTGCTT CAACTATACC AGTTATTTGG	79	60
	Campylobacter coli	TGTTCTTACT TCAAGAGATG GTAGAGGGAT TAAAATCACA GGTAGCATAG GTGTAGGAGC	79.5	60
	Campylobacter fetus	GAAACTACTC GCAAATTTTA AGGCTCAAAA ATGATAAACG CTAAACTCAT AGATCACATC TT	78.4	62
	Campylobacter jejuni	CGAAGGTATC ATCATAAGTT TAAATGCTTA TGCAACCATA CTAGGACAAG AAATCACACT CG	79.9	62
	Campylobacter upsaliensis	TAAGGGTAAT ATTATCGAGG AATTTGTAGA GGCAAGGCAA GATGGCGAAA CGATTC	81.6	56
	Enterohemorrhagic *Escherichia coli* (EHEC)	Refer to *eae* and *stx1* probes		
	Enteroinvasive *Escherichia coli* (EIEC)	(ipaB) GATTATCCGA ACTCGA CCCAGATTCACCAG AAAAT AAAAAATTAAGACGGGGAG AAATAC	80.9	60
		(ipaD) TTATTACATT CAGCCCCG AAAGAAGCTGAGCTTGATGGAT ATGAAATGAT ATCTCATAGA	80.9	60
	Enteropathogenic *Escherichia coli* (EPEC)	(eae) CATGAAGACT ATATCTATAA CATCCACACA ATAAAAAACC CTCCGAAGAG GGGGAAGAGG	81	60
	Shiga toxin-producing *Escherichia coli* (STEC)	(stx1) ACAAATAATG TTTTTTATCG CTTTGCTGAT TTTTCACATG TTACCTTTCC TGGTACAACT	79.2	60
		(stx2) AAATACTTTC TACCGTTTTT CAGATTTTAC ACATATATCA GTGCCCGGTG TGACAACG	80.4	58
	Grimontia hollisae	AAGGTAATTA GAAGTGAAAT TATCAAGGAC GTTTATAACC AACCCCTTCA CCCTGGCC	81	58
	Klebsiella oxytoca	ACTTATCACT CTCAAGGAAT CAGAAATGAT AAAAAGTTCG TGGCGTAAAA TTGCAATGCT	81.1	60
	Laribacter hongkongensis	GAACTGGGCT CTGGAAGAGT AAGCTGCATA TTTGTGGTAT ACAAATATAT CGTTGTTTTA	78.8	60
	Listeria monocytogenes	AGCATCCATT TACATTACAT AAAAAGGGGG GGTACTAGTG CAATCAATTG AAGACATCTG	81	60

Table 1 *(continued)*

	Bacteria	Sequences	TM (°C)	Length (bp)
	Salmonella enterica	ACATGAACAA GTTTCGGAAT GTGATCAATT TAAAAATTTA TTGACTTAGG CGGGCAGATA	80.9	60
	Shigella sonnei	ATTTATATCG GCGTAATATT ATCAGTCGTT ATTATCTCAG GTACGGGATA TGGTAGATGC AC	78.3	62
	Tropheryma whipplei	TAGCCATCTT GCCTCTGTTA TGGATGATAT TGAGGTATAC GATGCAACAA AAAAGACTAT T	80.1	61
	Vibrio alginolyticus	TTGTTTGTTC TCTCATTCGT ATTATTTATT TCAAGTACAT CATGTCTTCT GGCTGGAGTT A	78.6	61
	Vibrio cholerae	AAGGTTCCTT TTTGTAGAGG TGGGGAAAAG TGCATGTTTC TCTTCTTATT CATAGCCAAT	81.3	60
	Vibrio parahaemolyticus	AAATCTCCAG AGTTTGTTAA AACCGTTCCA AAACGAGGCT ATCAACTCAT TTGTACTGTT	80.8	60
	Vibrio vulnificus	CTTAATAACA AAAATAGAAA TGTAGGACGC CTTACCCTAC TCTGCTGTTT GTTTGCGGC	80.7	59
	Yersinia enterocolitica	TTTTTTAGAA AAGGGACAGT TTGTACAAGT TTTCGGCCTA ACAATAAAAC CAAACAAGCC	80.4	60
Intestinal microbiota	*Gemmata obscuriglobus*	TAGATAGTAG ACCCAGATAT GGGTTTACTG TCGAAGTTAA AATGCTAAGT ACCCCGCCTG	80.2	60
	Pirellula staleyi	ATCCCTAGAT TCCCTAATTA TTGCATACTG AATCCATAGG TATGCAAGGC CAACCCAG	81.9	58
	Planctomyces brasiliensis/maris	AAGCGACTTT TTCAATCATT TTTGAAAGAG TTTTTTGCTT GCTGAGTGAA ACACTCG	81.9	57
	Planctomyces limnophilus	ATTTTCTCGA TAATACGCGG GTGATACGCG AAGAGTTTCT ACATACATTT ACCGAACT	80.7	58
	Rhodopirellula baltica	AAGAACCTTA TCCTAGACTT GACATGCTTG AGAATCCCTA TGAAAGTAGA GAGTGCCCTT	80.3	60
Internal control	*Methanobrevibacter smithii*	CCTCCAACAT TAAAAGGTCG TGAAACTTTA ACATGGCCAT CATGTATTAA ATAGAAAGGA	80	60

supernatant was collected to avoid fecal debris, and glass beads (size <106 μm; Sigma Aldrich, Saint-Quentin Fallavier, France) were added to grind the specimen using the FastPrep® apparatus (MP Biomedicals, Illkirch, France) at 6.5 m/s for 90 s. This step was repeated once. A total of 25 μL of proteinase K (Qiagen, Courtaboeuf, France) and 180 μL of lysis buffer provided by the Nucleospin Tissue kit (Macherey Nagel, Hoerdt, France) were added before overnight incubation at 56 °C. Next, 100 μL total DNA was extracted from 200 μL specimen using the EZ1 DNA Tissue kit (Qiagen, Courtaboeuf, France). Extracted DNA was further purified using a phenol-chloroform protocol (33). Each extracted specimen was analyzed with a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, USA) to evaluate DNA amounts. The non-diarrheal stool specimens were not lyophilized because we used lyophilization to concentrate diarrheal stool where the pathogens could be in low inoculums to avoid the dilution effect.

Table 2. Probe sequences targeting viruses

Viruses	Sequences	TM (°C)	Length (bp)
Adenovirus	AAAACAAAAC AAACTCCTTT GGACAAGCTC CCTATATAGG ACAAAAAATC ACCAATCAGG	80.9	60
Astrovirus	TTAAGCCTGG GAAGGTCATC TGTAGTGACA GTATAGTTGG GTTATCCTTT TGTGGCTT	81.4	58
Bocavirus	AATTGAGTAT TAAACCTATA TAAGCTGCTG CACTTCCTGA TTCAATCAGA CTGCATCC	79.3	58
Hepatitis A virus	TAATACTTCT ATGAAGAGAT GCTTTGGATA GGGTAACAGC GGCGGATATT GGTGAGTTAT	80.4	60
Norovirus	GGAGAAGCCT CACTCCATGG TGAAAAATTT TACAGGAAAA TATCTAGCAA AGTCATACAT	79.8	60
Rotavirus	AAAGGAATTG ATCAAAAGAT GAGAGTACTT AATGCTTGCT TTAGTGTGAA AAGAATACCA GG	78.7	62
Calicivirus	AACCACTCCC CAGGTAGCTC AAATGTTTAA ATTTTATTTC CTTAACTGTG ATGCCACAC	81.3	59

Table 3. Real-Time PCR system use for the specific detection of *Salmonella enterica*, *Escherichia coli*, adenovirus and *Methanobrevibacter smithii*

Microorganisms	Sequences	Length (bp)
Salmonella enterica	CAAGAAATACCTGGCGGAAA	20
	CGGGACAAAAGAACGGATTA	20
	GTTCGGCATCGAAATCCGCG	20
Escherichia coli	GCTGCGCGTGCAAATGCG	18
	CATGGTCATCGCTTCGGTCT	20
	CATCAGAAACTGAACACCAC	20
Methanobrevibacter smithii	GCGCGAACCGGATTAGATAC	20
	GCGACCGTACTTCCCAGG	18
	CGATGCGGACTTGGTGTTGGGG	22
Adenovirus	GCCACGGTGGGGTTTCTAAACTT	23
	GCCCCAGTGGTCTTACATGCACATC	25
	TGCACCAGACCCGGGCTCAGGTACTCCGA	29

PCR and real-time PCR

In parallel with the DNA microarray experiment, each stool specimen was tested with real-time PCR for the specific detection of *S. enterica*, EHEC, EIEC, EPEC, STEC, adenovirus, and *M. smithii*. Primers and probes (Table 3) were diluted to 20 pmol/μL and 25 pmol/μL, respectively. PCR mixtures (20 μL) contained 10 μL Master Mix (Qiagen), 0.5 μL each primer, 0.5 μL uracil-DNA-glycosylase (UDG) (Invitrogen-Life Technologies, Saint Aubin, France), 4 μL water, and 4 μL DNA. Real-time PCR conditions included 2 min of UDG decontamination at 50 °C and 10 min of denaturation at 95 °C, followed by 40 cycles of 1 s at 95 °C, 35 s at 60 °C and 45 s at 72 °C. Each specific real-time PCR assay included a positive and a negative control. The cut-off for positivity was established at 38 cycle threshold (Ct). All the specimens were tested in duplicate. The extraction of *C. jejuni* was validated by classical PCR using two specific pairs of primers. The first pair targeted the *fla* gene (34), and the second one targeted the *wlaC* gene (35). These primer pairs were designed in our laboratory and generated 3 390– and 600–bp fragments, respectively. Each PCR was performed in a 25-μL mixture containing 2.5 μL of 10 × buffer (Qiagen), 0.5 μL of each primer, 2.5 μL of deoxynucleotide triphosphate mix (Euromedex, Souffelweyersheim, France), one unit of Hot Start (Qiagen), 10.8 μL water and 5 μL DNA. PCR was performed under the following conditions: a 5-min denaturation at 95 °C; 40 cycles of 30 s at 95 °C, 2 min at 60 °C and 1 min at 72 °C; and a final extension step of 5 min at 72 °C for the *fla* gene; and denaturation for 5 min at 95 °C; 40 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C; and a final extension step of 5 min at 72 °C for the *wlaC* gene.

DNA microarray assay

The Genomic DNA ULS Labeling Kit™ and the ULS-Cy™3 reagent were used according to the

supplier's instructions for an 8 × 15K microarray (Agilent Technologies). This protocol allowed for labeling 10 μL of DNA. Hybridization was then performed according to the Agilent protocol by adding 25 μL of reaction mixture [2 μL of Cot-1 DNA 1.0 mg/mL (Life Technologies), 0.5 μL of Agilent 100X Blocking agent, and 22.5 μL of Agilent 2X Hi-RPM hybridization buffer] to each labeled DNA specimen. Specimens were then incubated at 95 °C for 3 min and 37 °C for 30 min. In total, 11 μL of Agilent-CGHblock was added to each specimen and hybridized in a total volume of 45 μL at 65 °C for 40 h. All of the samples were hybridized in duplicate on our microarray. The background value was fixed at four fluorescence units, and the positivity threshold was set at nine fluorescence units. A positive detection was defined by over two-thirds of the specific probes exhibiting a fluorescence value higher than nine. Fluorescence intensity values were expressed as the mean of intensities measured for all homologous positive probes. All data were then normalized using 'R' software, available online at http://cran.r-project.org/doc/manuals/R-admin.htmL#Top.

Multiplexed detection

To test the capacity of the DNA microarray to simultaneously detect several pathogens in one stool specimen, we collected a stool sample that was naturally infected by adenovirus. An aliquot of this stool specimen was spiked with 10^4–10^6 CFU/mL (final concentration) *C. jejuni* CIP 70.2 in PBS.

RESULTS

PCR and real-time PCR

The DNA extraction protocol used in this article yielded 41 ± 28 ng/mL total DNA.

M. smithii DNA was detected in the nine negative control stool specimens (Ct mean value, 30.14), in the stool sample spiked with *S. enterica* (Ct value, 34.18) and in the 40 pathological stools (Ct values, 21.18 to 31.23).

S. enterica DNA was not detected in the negative control stool specimens, but it was detected in the stool sample spiked with *S. enterica* (Ct value, 19.46). Thirty *S. enterica*-infected diarrheal stools were lyophilized before DNA extraction. The real-time PCR detection of *S. enterica* was positive in all specimens, with Ct values between 24.31 and 29.47. *S. enterica* was not detected in the remaining ten pathological stool specimens.

Regarding pathogenic *E. coli*, none of the five targets were detected in the negative controls or the positive control. The *ipaB* gene was detected in one pathological stool infected with *C. jejuni* (Ct value, 32.61). One pathological stool sample infected with EPEC was positive for the *stx1* gene (Ct value, 22.78). The *stx2* gene was detected in two pathological stools infected with *C. jejuni* (Ct values, 29.08 and 33.40, respectively). The *ipaD* and *eae* genes were negative for all stool samples tested.

Four adenovirus-contaminated stool specimens yielded Ct values between 16.43 and 21.68; adenovirus was not detected in the other pathological stools or control stools.

Four *C. jejuni*-infected pathological stool specimens yielded positive results for *fla* and *wlaC* genes, while the negative and positive controls and the remaining pathological stool specimens were negative.

DNA microarray detection

The *M. smithii* internal control was detected in 47/49 (95.9%) stool specimens tested, with fluorescence signals between 9 and 14.5 units.

Twenty-nine of 30 (96.7%) *S. enterica*-infected pathological stool specimens yielded 194 positive *S. enterica*-specific probes, with fluorescence signals between 9 and 11.1; no other pathogen was detected in the 30 specimens, and *S. enterica* was not detected in the remaining stool specimens.

The pathological stool specimen infected with EPEC yielded 194 positive *stx1* gene probes, with a mean fluorescence value >10 units. The pathological stool contaminated with EHEC yielded 178 positive *eae* gene probes, with a mean fluorescence value of 10.4 units. The *ipaB* probe was positive in 13/47 (27.7%) remaining stools without a pathogenic *E. coli*. The nine control stools and the 38 remaining pathological stools were negative for all probes specific for pathogenic *E. coli*.

Four *C. jejuni*-contaminated pathological stool specimens yielded 132 positive probes, with a mean fluorescence of 9.1 in all specimens; the remaining specimens were negative for *C. jejuni*.

Four adenovirus-infected pathological stool specimens yielded positive detection, with fluorescence values between 9.1 and 10.9, and the

remaining specimens were negative for adenovirus. One of these pathological stools infected with adenovirus and spiked with *C. jejuni* yielded a positive detection of 10^5 and 10^6 *C. jejuni* CFU/mL with fluorescence values of 14.3 for adenovirus and 11.9 and 12.1 for *C. jejuni*, respectively; the 10^4 CFU/mL inoculum was not detected.

DISCUSSION

The results here obtained in a clinical microbiology laboratory, were interpreted as valid because all the negative controls remained negative in all of the experiments. In addition, DNA microarray data were controlled in parallel with real-time PCR, including the detection of *M. smithii* DNA as an internal positive control. Indeed, we previously showed that this archaeal DNA was detected in 95.7% of individuals (24), making this archaeon a suitable target to control for total DNA extraction and the absence of PCR inhibition in extracted stool specimens. Detecting *M. smithii* DNA was further used to confirm that the dilution of diarrheal stool specimens did not prevent the DNA-based detection of pathogens. In this study, we lyophilized diarrheal stools as lyophilization has previously been used to suppress PCR inhibition in animal stool specimens (36,37). We therefore recommend lyophilizing diarrheal stool specimens before detecting enteropathogenic DNA.

The DNA microarray reported in this article allowed for the simplex detection of enteropathogens in stool, with a sensitivity of 97.5%. However, detecting pathogenic *E. coli* was problematic. We designed probes based on published virulence genes reported to be specific for each pathogenic *E. coli*. EIEC strains were detected with *ipaB* and *ipaD* probes (30). *IpaB* is a gene encoding an invasion protein found in not only *E. coli* strains but also *Shigella* and *Salmonella* strains. This gene is known to be specific for these strains (38), but we found that our *ipaB* probe gave positive results for 13/47 (27.7%) stool samples tested. This result may be due to a lack of specificity of the probe despite our favorable in silico analysis. Alternatively, this observation could be explained by the fact that this gene is much more ubiquitous than previously reported. For example, only 6% of genes are common between all *E. coli* strains, which are called the core genome (39), and in fact, we do not really know the virulence genes that can reliably identify strains of *E. coli*.

Intestinal infections could be caused by several pathogens at the same time, but the simultaneous detection of enteric bacteria and viruses has never been performed using a DNA microarray. Developing a protocol for the multiplex detection of human enteric pathogens was challenging, but our data indicate that it is possible to achieve the multiplexed detection of some enteropathogens.

Previously reported DNA microarrays allowed for the detection of only a few bacteria (12, 13, 20). Regarding viruses, DNA chips have allowed for the detection of the rotaviruses A group (40–43). In 2009, a DNA microarray was designed for the detection of common foodborne viruses, including human rotaviruses (44); however, this system was not adapted for the diagnosis of human acute enteritis. No DNA microarray has been published for the dual detection of viral and bacterial enteropathogens.

Our data confirm the proof-of-concept of multiplex detection for enteric pathogens using a DNA microarray. Further studies will aim to reduce the turn-over time, which was 3 h in this study. The DNA microarray technique is amenable to automation and could be used for epidemiological studies and the selection of stool specimens devoid of any known pathogen for further investigations using additional approaches. The cost of DNA microarray remains a negative point as this technique in our laboratory is estimated at about 130 € per sample. In addition, a more complete version of the DNA microarray could be used for the repertoire of the gut microbiota using the protocol developed in this study.

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