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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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 *Gemmatrata obscuriglobus, Pirellula staleyi, Planctomyces brasilensis/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 0.073 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

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### Table 1. Probe sequences targeting bacteria

| Bacteria                          | Sequences                                                                 | TM (°C) | Length (bp) |
|----------------------------------|---------------------------------------------------------------------------|---------|-------------|
| **Intestinal pathogens**         |                                                                           |         |             |
| *Aeromonas caviae*               | TTGTATGGAT ACCTTTTTAG AACAATTTAA GTGTGGATTTC GATCGCATTC GTTGATTTCT       | 80.4    | 60          |
| *Arcobacter butzleri*           | ATATGAACCTT GTGCACTACGC TGTTCCTACGT CAACTATACG AGTTATTTGG                | 79      |             |
| *Campylobacter coli*            | TGGTCTCCTA CTAGAGATG GTAGAGGGAT TAAAATCACA GGTAGCATAG GTGTAGGAGC          | 79.5    | 60          |
| *Campylobacter fetus*           | GAAACTACTC GCAAAATTATA AGGCTCAAAAA ATGATACATTAC AGATACATC TT              | 78.4    | 60          |
| *Campylobacter jejuni*          | CGAAGGGTATC ATCATAAGTT TAAATGCTTA TGGACACATA CTAGAACAG AATACACACT CAAATC | 79.9    |             |
| *Campylobacter upsaliensis*     | TAAGGGTAAT ATATACGAGG AATTTGTAGA GGCAAGGCAA GATGCGAAA CGATTC             | 81.6    | 56          |
| **Enterohemorrhagic Escherichia coli (EHEC)** | Refer to eae and stx1 probes                                              |         |             |
| **Enteroinvasive Escherichia coli (EIEC)** | (ipaB) GATTATCCGA ACTCGA CCGAGTACCCAG AAAAT AAAAAATTAAGACGGGAG AAATAC (ipaD) TTAATACATT CAGCCCCG AAAGAAGGCTGAGCTTGATGGAT ATGAAATGAT ATCTCATAGA | 80.9    | 60          |
| **Enteropathogenic Escherichia coli (EPEC)** | (eae) CATGAAGACT ATATCTATAA CATCCACACA ATAAAAAACC CTCCGAAGAG GGGGAAGAGG | 81      |             |
| **Shiga toxin-producing Escherichia coli (STEC)** | (stx1) ACAAAATAAG TTTTTATCG CTTTGGCTAT TTTCACATG TTACCTTTCC TGGTACAACCT | 79.2    | 60          |
| **Grimontia hollisae**           | AAGGTAATTA GAAGTGAAAT TATCAAGGAC GTTTATACAC AAAACCCCTCA CCCCCTGGCC       | 81      | 58          |
| **Klebsiella oxytoca**           | ACTTATCAGT CTCAAGGAAT CAGAAATGAT AAAAAGTTTCG TGGCGTAAA TGGCAATTGCT      | 81.1    | 60          |
| **Laribacter hongkongensis**    | GAACCTGGAGC CTGGAAGAGT AAGCTGATA TTTGGTGGTAT ACAAATATAC CTTGGTGTATTA     | 78.8    | 60          |
| **Listeria monocytogenes**       | AGCATCACCATT TACATTACAT AAAAAGGGGG GTTAGCACTAGT CAATCAATTG AAACATCTG     | 81      | 60          |
supernatant was collected to avoid fecal debris, and glass beads (size \(<106\, \mu m\); Sigma Aldrich, Saint-Quentin Fallavier, France) were added to grind the specimen using the FastPrep/C226 apparatus (MP Biomedicals, Illkirch, France) at 6.5 m/s for 90 s. This step was repeated once. A total of 25 L of proteinase K (Qiagen, Courtaboeuf, France) and 180 L of lysis buffer provided by the Nucleospin Tissue kit (Macherey Nagel, Hoerdt, France) were added before overnight incubation at 56 °C. Next, 100 L total DNA was extracted from 200 L 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 1 (continued)

| Bacteria                  | Sequences                          | TM (°C) | Length (bp) |
|---------------------------|------------------------------------|---------|-------------|
| *Salmonella enterica*     | ACATGAACAA GTTTCGGAAT              | 80.9    | 60          |
|                           | GTGATCAATT TAAATAATTTA TGGCTTGGGAA |         |             |
|                           | TAGACCTTGG CCGGTCGTTA GCAGCTTGATAC |         |             |
| *Shigella sonnei*         | ATTTATATCG GGCATTIAATTT ATCACGTTTT | 78.3    | 62          |
|                           | ATTATCTCAG GTGACCTGATA TGGTATAGTA |         |             |
| *Tropheryma whipplei*     | TGGGCGTAT AGGATAGAC TGGTACCTGTT   | 80.1    | 61          |
|                           | GATGCAACAA AAAAAAGACAT TGGTCCCTCT |         |             |
| *Vibrio alginolyticus*    | TTGTTTGTTT TCTTACCTCG              | 78.6    | 61          |
|                           | ATTATTTGTT TCAAGTACAT CATGTCCTCT |         |             |
| *Vibrio cholerae*         | AAGGTTCCTT TTTGTAGAGG              | 81.3    | 60          |
|                           | TGGGGAAGAG TGCACTGTTTC TTCTTTATAT |         |             |
| *Vibrio parahaemolyticus* | AAATCTCCAG AGTTTTGTTAA             | 80.8    | 60          |
| *Vibrio vulnificus*       | CTTAATACAA AAAATAAGAAA TGTAGAGGC | 80.7    | 59          |
| *Yersinia enterocolitica* | TTTTATTAGAA AAGGGAAGT TTTGATAATGA | 80.4    | 60          |
|                           | TTTGCGGCTTA ACAATAAACG AAAAACAGGC |         |             |
| Intestinal microflora     | Gemmata obscuriglobus             | 80.2    | 60          |
|                           | TAGATAGTAG ACCAGATAT GGGTTTACTCT TCGAAATTTA |         |             |
|                           | AATGCTATAAT ACCCCGCTG TGCCACCTA |         |             |
| *Pirellula staleyi*       | ATCCCTGAT TCCCTAATTA                | 81.9    | 58          |
|                           | TGGGATACGT AATCCATAGG TATGGAAGGC |         |             |
|                           | CAAACGAGC TCTGACCTAT TATGGAAGGC |         |             |
| *Planctomyces brasiliensis/maris* | AAGCGACTTT TTCAATCACTT             | 81.9    | 57          |
|                           | TTGGAAAGAG TTTTTTTGCTT GCTGAGTGA AACACTG |         |             |
| *Planctomyces limnophilus*| ATTTTCTCGA TAATACGC | 80.7    | 58          |
|                           | GTGATACGGG AGAAGTTCCT ACATACATTT ACCGAACCT |         |             |
| *Rhodopirellula baltica*  | AAGAACCTTA TTCTAGACTT GACATTGTTAG AGAATCCCCTA | 80.3    | 60          |
|                           | TGAAGATAGA GAGTGCTCCT GAAGACCTCT |         |             |
| Internal control          | Methanobrevibacter smithii         | 80      | 60          |
|                           | CCTGCAACAT TAAAGGTCCG TGAACCTTTA ACATGGCCCAT CATGTTAAT ATAGAAGGA |         |             |

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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/L and 25 pmol/L, 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 3 390–442 and 600–490 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

### Table 2. Probe sequences targeting viruses

| Viruses         | Sequences                                                                 | TM (°C) | Length (bp) |
|-----------------|---------------------------------------------------------------------------|---------|-------------|
| Adenovirus      | AAAACAAACAC AAACCTCTTTT GGAACAGCCTC                                       | 80.9    | 60          |
|                 | CAGAGAAAATTACCTGGCGGAAA                                                   | 80.9    | 60          |
|                 | GCGGGACAAAAGAACGGATTA                                                    | 20      |             |
|                 | GTTGGCATACTGGAAATCCGCG                                                   | 20      |             |
|                 | GCTGCGCGTGCAAAATGCG                                                     | 18      |             |
|                 | CATGGTATCGCTTCAGGTCT                                                    | 20      |             |
|                 | CATCAGAAACTGAACACCAC                                                    | 20      |             |
|                 | GCCACGGTGAGTTCAATGAC                                                     | 23      |             |
|                 | CAGATCCGCTTCCTCCAGATCC                                                  | 25      |             |
|                 | GGACGCACTTCCAGATCC                                                     | 29      |             |
|                 | GGCAGCGGGATGATGGG                                                       | 80.4    | 60          |
|                 | ATATGATGATGGG                                                           | 79.8    | 60          |
|                 | AAAAGAAATTTG ATCAAAAGAT GAGAGTACTT                                     | 78.7    | 62          |
|                 | ATACGTCTCT TTAGTGTGGA AAGAATACCA GG                                      | 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*           | CAAGAAATACCTGGCCGGAAA                                                    | 20          |
|                                  | CAGAGAAAATTACCTGGCGGAAA                                                   | 80.9        |
|                                  | GCGGGACAAAAGAACGGATTA                                                    | 20          |
|                                  | GTTGGCATACTGGAAATCCGCG                                                   | 20          |
| *Escherichia coli*              | GCTGCGCGTGCAAAATGCG                                                     | 18          |
|                                  | CATGGTATCGCTTCAGGTCT                                                    | 20          |
|                                  | CATCAGAAACTGAACACCAC                                                    | 20          |
| *Methanobrevibacter smithii*    | GGCAGCGGGATGATGGG                                                       | 80.4        |
|                                  | ATATGATGATGGG                                                           | 79.8        |
|                                  | AAAAGAAATTTG ATCAAAAGAT GAGAGTACTT                                     | 78.7        |
| *Adenovirus*                    | GCCACGGTGAGTTCAATGAC                                                     | 23          |
|                                  | GGCAGCGGGATGATGGG                                                       | 25          |
|                                  | GGACGCACTTCCAGATCC                                                     | 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).
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 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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