Genetic Analysis and Detection of fliC_H1 and fliC_H12 Genes Coding for Serologically Closely Related Flagellar Antigens in Human and Animal Pathogenic Escherichia coli

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The E. coli flagellar types H1 and H12 show a high serological cross-reactivity and molecular serotyping appears an advantageous method to establish a clear discrimination between these flagellar types. Analysis of fliC_H1 and fliC_H12 gene sequences showed that they were 97.5% identical at the nucleotide level. Because of this high degree of homology we developed a two-step real-time PCR detection procedure for reliable discrimination of H1 and H12 flagellar types in E. coli. In the first step, a real-time PCR assay for common detection of both fliC_H1 and fliC_H12 genes is used, followed in a second step by real-time PCR assays for specific detection of fliC_H1 and fliC_H12, respectively. The real-time PCR for common detection of fliC_H1 and fliC_H12 demonstrated 100% sensitivity and specificity as it reacted with all tested E. coli H1 and H12 strains and not with any of the reference strains encoding all the other 51 flagellar antigens. The fliC_H1 and fliC_H12 gene specific assays detected all E. coli H1 and all E. coli H12 strains, respectively (100% sensitivity). However, both assays showed cross-reactions with some flagellar type reference strains different from H1 and H12. The real-time PCR assays developed in this study can be used in combination for the detection and identification of E. coli H1 and H12 strains isolated from different sources.

Keywords: E. coli, molecular serotyping, fliC type H1 gene, fliC type H12 gene, STEC, ExPEC

INTRODUCTION

Strains belonging to the species of Escherichia coli are ubiquitous as commensals in the gut of humans and warm-blooded animals. Apart from their role as beneficial microbes, some E. coli strains are known to behave as human and animal pathogens, causing a wide spectrum of extraintestinal and enteric diseases, with urinary tract infection and diarrhea as most frequent (Kaper et al., 2004; Stenutz et al., 2006). Pathogenic and apathogenic E. coli cannot be discerned from each other by their morphology, cultural properties or fermentation reactions. As a consequence, serotyping is used since the 1940s as a diagnostic tool for identification of animal and human pathogenic E. coli strains (Orskov and Orskov, 1984).

E. coli serogroups are commonly defined by the antigenic properties of the lipopolysaccharide which is part of the outer membrane (O-antigen) (Stenutz et al., 2006). Motile E. coli strains can be
additionally typed for their flagellar filaments (H-antigen) (Orskov and Orskov, 1984). *E. coli* O- and H-antisera are usually produced by immunization of rabbits with respective reference strains (Orskov and Orskov, 1984; Edwards and Ewing, 1986). At present, 182 O-antisgens and 53 H-antisgens have been described (Scheutz et al., 2004; Scheutz and Strockbine, 2005). The resulting O:H serotype (for example O157:H7) is commonly used for describing *E. coli* isolates (Bettelheim, 1978; Orskov and Orskov, 1984).

Complete serotyping of *E. coli* is laborious and time-consuming and performed only in a few specialized reference laboratories worldwide. Moreover, cross-reactivity which is observed between some *E. coli* O-groups and H-types can complicate the interpretation of serotyping results. Last but not least, serotyping fails if autoagglutinating (O-antigen or H-antigen rough) and non-motile (NM) *E. coli* strains have to be examined (Orskov and Orskov, 1984; Edwards and Ewing, 1986). For these reasons, attempts were made to substitute serotyping by molecular typing of O-antigen and H-antigen encoding genes.

In the recent years, the nucleotide sequences of all known O and H-antigen genes in *E. coli* have been elucidated (Wang et al., 2003; Iguchi et al., 2015a). Molecular methods such as PCR and nucleotide sequencing have been successfully employed for typing of O- and H-antigen genes in *E. coli* (Beutin and Fach, 2014; Joensen et al., 2015; Iguchi et al., 2015b). Molecular serotyping was shown to be specific and sensitive and can substitute conventional serological detection of *E. coli* surface antigens (Bugarel et al., 2010; Fratamico et al., 2011; Clotilde et al., 2015; Iguchi et al., 2015b; Joensen et al., 2015). In contrast to serotyping, molecular detection of O- and H-antigen genes is easier and faster to perform and O-rough and non-motile strains can be typed on the basis of their O- and H-antigen genes (Beutin and Fach, 2014; Joensen et al., 2015).

We have previously investigated the genetic variability of flagellar types H19, H25 and H28 in *E. coli* (Beutin et al., 2015a,b). These flagellar types are widespread in strains belonging to numerous O-serogroups but are also associated with enterohemorrhagic *E. coli* O145:H25, O145:H28, and O121:H19 strains. By nucleotide sequence analysis of *fliC* (flagellin) genes encoding H19, H25, and H28 flagella we have observed a high genetic variability among *fliC* 

| O1-O181 and H-types H1-H56 (Orskov and Orskov, 1985; Johnson et al., 1994, 2005, 2006; Olesen et al., 2009) Adherent-invasive *E. coli* (AIEC) O83:H1 

strains were associated with Crohn’s disease in human patients (Allen et al., 2008; Nash et al., 2010) and flagellar type H1 is associated with biofilm formation and invasive properties of AIEC strains (Eaves-Pyles et al., 2008; Martinez-Medina et al., 2009) as well as with intestinal colonization (Martinez-Medina and Garcia-Gil, 2014). Moreover, H1-type flagellum is a characteristic trait of Shiga toxin 2e-producing *E. coli* O139:H1 strains which are a major cause of edema disease in pigs (Tschape et al., 1992; Frydendahl, 2002; Fairbrother et al., 2005; Beutin et al., 2008). Conversely, the flagellar type H12 has not been associated with pathogenic *E. coli*, except from human enterotoxigenic O78:H12 and O128:H12 strains (Orskov and Orskov, 1977; Echeverria et al., 1982; Shaheen et al., 2004).

In this work we have analyzed the nucleotide sequences of *E. coli* H1 and H12 strains in order to detect characteristic *fliC* sequence alterations corresponding with these closely related H-types. Subsequently, we have developed a real-time PCR procedure for reliable discrimination of H1 and H12 flagellar types in *E. coli*. The protocol should be useful for diagnostic and epidemiological investigations of human and animal pathogenic strains of *E. coli*.

**MATERIALS AND METHODS**

**Bacteria**

*E. coli* strains used in this study were derived from the collections of the National Reference Laboratory for *E. coli* (NRL *E. coli*) at the Federal Institute for Risk Assessment (BfR) in Berlin, Germany and from the French Agency for Food, Environmental and Occupational Health and Safety (Anses) in Maisons-Alfort, France. *E. coli* strains used for specificity study included in particular the *E. coli* reference strains belonging to serogroups O1-O181 and H-types H1-H56 (Orskov and Orskov, 1984; Edwards and Ewing, 1986). All strains have been previously described for their serotypes and for virulence genes associated with STEC (Beutin et al., 2015a,b). All strains were grown overnight at 37°C in Luria broth, and DNA was extracted according to manufacturers instructions using InstaGene matrix (BioRad laboratories, Marnes-La-Cooquette, France).

Real-time PCR assays were performed with an ABI 7500 instrument (Applied Biosystems, Foster City, CA, USA) in 25-µl reaction volumes, a LightCycler Nano (Roche Diagnostics,
Meylan, France) in 10 µl reaction volumes or with a LightCycler 1536 (Roche Diagnostics, Meylan, France) in 1.5-µl reaction volumes according to the recommendations of the suppliers. Primers and TaqMan probes were used at 300 nM final concentrations. The following thermal profile was applied to all instruments: enzyme activation at 95°C for 1–10 min as recommended followed by 40 cycles of denaturation at 95°C and annealing at 60°C.

**PCR Detection and Mapping of E. coli O-Antigen and H-Antigen Genes**

Mapping of fliC gene variants to their respective H-types was performed as previously described (Beutin et al., 2015a,b). Nucleotide sequence data obtained from thirteen fliC H1 and eight fliC H12 genes were used for designing TaqMan® real-time PCR probes and XS probes (minor groove binder replacement, Biolegio, Nijmegen, The Netherlands) and primers for specific detection of all genetic variants of thirteen fliC H1 and eight fliC H12 genes (this work). Real-time PCR probes and primers used in this work were designed with the software Primer Express V3.0 (Applied Biosystems) and are described in Table 1.

**Nucleotide Sequencing**

The nucleotide sequence of the PCR products were determined as described (Beutin et al., 2015b) and analyzed with the Accelrys DS Gene software package (Accelrys Inc., USA). The nucleotide sequences of the respective products for fliC homologs were determined and have been submitted to European Nucleotide Archive (ENA). The GenBank Accession numbers are listed in Table 2.

**RESULTS**

**Sources and Properties of E. coli H1 and H12 Strains**

The E. coli H1 and H12 strains investigated in this study were from human, animal, food, and environmental sources (Table 3). The thirty-one flagellar type H1 strains were associated with 10 different E. coli O-serogroups, O-rough and O-untypable strains and originated from healthy and diseased humans and animals and from food. The thirty-eight H12 strains divided into thirteen different O-groups of E. coli, and in O-untypable and O-rough strains. The H12 strains were from healthy and diseased humans and animals, from food and the environment. Production of Shiga-toxins (Stx) was found in 16 (42.1%) of the H12 strains and associated with five different O-groups. Fourteen (45.2%) of the E. coli H1 strains produced Stx, however most of these were from pigs with edema disease (O139:H1, Or:H1) and harbored the stx2e gene. O:H types known to be associated with E. coli causing extraintestinal infections of humans (O2:H1, O4:H1, O6:H1, O25:H1) were detected among the investigated H1 strains. Interestingly, strains belonging to these serotypes originated not only from humans but also from animals and food. Certain strains belonged to serotypes which have not been previously associated with clinical disease and their role of pathogens for humans and animals is not yet known.

**Nucleotide Analysis of E. coli fliC H1 and fliC H12 Genes**

The nucleotide sequences of the reference strains (Orskov and Orskov, 1984) for E. coli flagellar antigens H1 (strain Su124, Genbank accession AB028471.1) and H12 (Bi 316–42, GenBank accession AJ249997) (Wang et al., 2003) have been published previously. The length of coding sequence of each fliC H1 and fliC H12 gene is 1788 nucleotides and both sequences have 97.5% identity (44 nucleotide exchanges) at the nucleotide level and 98.98% identity and 99.16% similarity at the amino acid level (7 amino acids (aa) exchanges). Additional fliC nucleotide sequences from six E. coli H1 and five E. coli H12 strains were obtained in this work (Table 2). These sequences were compared with seven fliC H1 sequences and three fliC H12 sequences already available in GenBank (Table 2). All 21 H1 or H12 flagellin genes have a 1788 nucleotides length that codes for 595 amino acid residues.

A cluster analysis performed with thirteen fliC H1 and eight fliC H12 sequences is shown in Figure 1. Four different genotypes were detected among the thirteen fliC H1 strains. Uropathogenic E. coli O2:H1, O6:H1, O25:H1, and AIEC O83:H1 strains were identical for their fliC H1 sequences and assigned to a large cluster composed by eight strains. A smaller cluster was formed by six fliC H1 strains; four of these were Stx2e producing O139:H1 causing edema disease in pigs.

Six different genotypes were found among the eight fliC H12 strains. Identical fliC H12 sequences were only found between two O9:K9:H12 strains and each one O55:H12 and O153:H12 strain, respectively.

### Table 1 | Primers and probes for real-time PCR detection of E. coli flagellar types H1 and H12.

| Target gene | Forward primer, reverse primer and probe sequences (5′-3′)a | Length and location within Table 2 (5′-3′) |
|-------------|-------------------------------------------------------------|-------------------------------------------|
| fliC H1     | AGGAGAAGGAAATTCCGAGTCTT                                      | 338–369b                                    |
|             | AGGAGAAGGAAATTCCGAGTCTT                                      | 422–444c                                    |
|             | [6FAM]–GACCCGCGATCTGCGACAAAG                                   | 370–392d                                    |
| fliC H12    | TCGATTGGAGAATTCCGAGTCTGAGT                                      | 331–353b                                    |
|             | CCGAAGAATTCCGAGTCTGCGACAAAG                                    | 402–421c                                    |
|             | [6FAM]–GTATCTGGCCACG                                         | 376–392d                                    |
| fliC H1/H12 | TGGATGAGAATTCCGAGTCTGAGT                                      | 1329–1353d                                  |
|             | GGTAGAATTCCGAGTCTGAGT                                          | 1395–1419d                                  |
|             | [6FAM]–CGAAGAATTCCGACG                                         | 1363–1393d                                  |

aXS probes (MGB-replacement) were used for fliC H1 and fliC H12, specific real-time PCRs.

bForward primer conserved in all analyzed fliC H1 and fliC H12 sequences.

cFliC H1 reverse primer: one mismatch at position 429: fliC H1 = G, fliC H12 = A (underlined).

dFliC H1 probe: one mismatch at position 381: fliC H1 = C, fliC H12 = T, and position 384: fliC H1 = C; fliC H12 = G (underlined).

eFliC H12 reverse primer: one mismatch at position 402 with fliC H1 = T (4/13 strains), fliC H12 = G (underlined).

fFliC H12 probe: one mismatch at position 381: fliC H12 = T, fliC H1 = C; and position 384: fliC H1 = T (4/13 strains), fliC H12 = C (underlined).

gConserved in all 21 fliC H1 and fliC H12 sequences from Table 2.
Amino acid Alterations between Flagellar Antigens H1 and H12 in *E. coli* Strains

An alignment of the amino acid sequences of thirteen *fliC*<sub>H1</sub> and eight *fliC*<sub>H12</sub> strains is shown in Table S1. All translation products had a length of 595 amino acids (aa). The eight *fliC*<sub>H12</sub> strains were showing only few alterations with one or more of the strains at aa positions 249, 258, 339, and 472 (99.2% similarity) (Table S1), generating six different protein sequences (Figure 2). The thirteen *fliC*<sub>H1</sub> strains split into three protein sequences (Figure 2) differing at positions 258, 431, and 481 (99.5% similarity) (Table S1). The aa changes were all located in the variable region of the *fliC* encoding flagellar antigen specificity (Wang et al., 2003). Differences in the aa sequence which could distinguish between all investigated *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> strains, respectively, were found at positions 302 (Glu/Lys), 340 (Asn/Lys), 361 (Gly/Asp), 391 (Thr/Lys), 396 (Asn/Asp), and 430 (Asn/Lys). The six flagellar type specific aa sequence differences were all located in the variable region of the *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> genes.

Development and Evaluation of Real-Time PCR Assays for Identification of *E. coli* *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> Strains

The close similarity between *E. coli* *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> translation products explains the serological cross-reactivity which was previously described for H1 and H12 antigens (Orskov and Orskov, 1984; Edwards and Ewing, 1986). As specific differences were found that distinguish between *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> sequences, molecular detection of the respective *fliC* genes could be more suitable than serotyping for clear identification of H1 and H12 strains of *E. coli*.

Based on the sequence data obtained for *E. coli* *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> genes we developed a TaqMan real-time PCR assay for common detection of *fliC*<sub>H1</sub> and *fliC*<sub>H12</sub> genes as well as
real-time PCR assays for specific detection of \( fliC_{H1} \) and \( fliC_{H12} \), respectively (Table 1). Short-length XS-probes (minor groove binder replacement) had to be employed to develop real-time PCR assays specific for \( fliC_{H1} \) and \( fliC_{H12} \) sequences (Table 1). We used two nucleotide substitutions between the sequences of \( fliC_{H1} \) and \( fliC_{H12} \) to design specific probes (Table 1).

The assays were first tested for sensitivity and specificity on 31 \( E. coli \) H1 and 38 \( E. coli \) H12 strains (Table 4) as well as on the \( E. coli \) H-type reference strains (H1-H56) (Orskov and Orskov, 1984; Edwards and Ewing, 1986). The real-time PCR for common detection of \( fliC_{H1} \) and \( fliC_{H12} \) reacted with all tested \( E. coli \) H1 and H12 strains (Table 4) and not with any of the reference strains encoding all other flagellar antigens than H1 and H12.

The \( fliC_{H1} \) and \( fliC_{H12} \) gene specific assays detected all \( E. coli \) H1 and all \( E. coli \) H12 strains, respectively (Table 4). However, both assays showed cross-reactions with some flagellar type reference strains different from H1 and H12. With the \( fliC_{H1} \) real-time PCR, cross-reactions were observed with H6, H7, H15, H20, H34, H37, H41, H45, H49, and H52 strains. The \( fliC_{H12} \) specific PCR reacted also with H7, H28, H31, and H41 strains (Table 5). Although the overall sequences of the \( fliC \) genes of H-types cross-reacting with the \( fliC_{H1} \) and \( fliC_{H12} \) real-time PCR are widely different from those of \( fliC_{H1} \) and \( fliC_{H12} \), they show high local similarities with the primers and probes sequences. In cases of cross reactivity, no or only minor differences (0–3 mismatches) were found between target-sequences and \( fliC_{H1} \)
TABLE 3 | Source and origin of E. coli H1 and H12 strains.

| Serotype  | Nos. of strains | Source | Origin/References |
|-----------|-----------------|--------|------------------|
| O2:H1     | 1               | Calves feces, diarrhea | Germany, 2010 |
| O2:K2:H1  | 1               | Human blood             | Orskov and Orskov, 1984 |
| O4:H1     | 1               | Raw milk cheese         | Germany, 2010 |
| O6:H1     | 4               | Pig feces, diarrhea (1), Human (3) | Germany, 2011, 2009 |
| O6:K5:H1  | 1               | Human feces             | Reister et al., 2014 |
| O15:H1    | 3               | Rabbit (2)              | Switzerland, 2007 |
| O22:H1    | 2               | Goat cheese (1)         | Orskov and Orskov, 2012 |
| O25:H1    | 1               | Human peritonem (1)     | Orskov and Orskov, 1984 |
| O27:H1    | 1               | salad                   | Germany, 2009 |
| O79:H1    | 1               | hare                    | Germany, 2007 |
| O9:K9:H12 | 11b             | Pig feces/organs, edema disease | Orskov and Orskov, 1984: Wild boar feces/organs, edema disease |
| O11:H12   | 1               | Human urine             | Orskov and Orskov, 1984 |
| O149:H1   | 2c              | Beef                    | Germany, 2011 |
| O153:H12  | 3               | Human peritonem (1)     | Orskov and Orskov, 1984 |
| O118:H12  | 1b              | Pig feces, edema disease | Germany, 2014 |
| O11:H12   | 1               | Pig feces               | Germany, 2007 |
| O9:K9:H12 | 5               | Human peritonem (1)     | Orskov and Orskov, 1984 |
| O15:H1    | 3               | Milk, beef              | Martin and Beutin, 2011 |
| O79:H1    | 1               | Human (1)               | Geser et al., 2012 |
| O22:H1    | 2               | Cattle feces            | Germany, 2010 |
| O49:H12   | 1               | Human urine             | Orskov and Orskov, 1984 |
| O65:H12   | 2b              | Milk, beef              | Martin and Beutin, 2011 |
| O79:H12   | 1               | Surface water           | Germany, 2011 |
| O98:H12   | 1               | Pork                    | Germany, 2013 |
| O104:H12  | 2               | Human, diarrhea         | Miko et al., 2013 |
| O118:H12  | 3d              | Human, diarrhea         | Pierard et al., 1998, Beutin et al., 2004 |
| O136:H12  | 4f              | Milk                    | Martin and Beutin, 2011 |
| O153:H12  | 3a              | Cattle feces            | Martin and Beutin, 2011 |
| O157:H12  | 3               | Human                    | Germany, 2007 |
| ONT:H12   | 2               | Pig                      | Kaufmann et al., 2006 |
| Or:H12    | 2               | Milk                     | Germany, 2014 |
| Or:H12    | 2               | Cattle feces             | Germany, 2010 |

<sup>a</sup>This list includes serotype reference strains: Nissle 1917 (O6:K5:H1) (Reister et al., 2014), EH550 (Or118:H12), Schoetz et al., 2012), Su 1242 (O2:K2:H1), E14a (O22:H1), CDC 63-57 (O139:H1), Bi316-42 (O9:K9:H12), U12-41 (O49:H1) (Orskov and Orskov, 1984).<sup>b</sup>Positive for strI2.<sup>c</sup>Positive for strI2d.<sup>2</sup>Positive for strI2e.<sup>3</sup>Positive for strI2f.<sup>4</sup>Positive for strI2g.

TABLE 4 | Detection of different E. coli H1 and H12 strains belonging to different O-serogroups by flcH1, flcH12 and flcH1/H12 Real Time PCR assays.

| Serotype  | Nos. of strains | CT-values<sup>a</sup> | CT-values<sup>a</sup> | CT-values<sup>a</sup> |
|-----------|-----------------|-----------------------|-----------------------|-----------------------|
| O2:H1     | 1               | 21.1                  | –                     | 22.2                  |
| O2:K2:H1  | 1               | 21.1–24.3             | –                     | 22.2–22.9             |
| O4:H1     | 1               | 24.7                  | –                     | 27.1                  |
| O6:H1     | 5               | 21.1–24.3             | –                     | 21.9–25.3             |
| O15:H1    | 3               | 19.6–25.5             | –                     | 21.2–24.0             |
| O22:H1    | 2               | 19.6–22.0             | –                     | 22.6–24.2             |
| O25:H1    | 1               | 22.5                  | –                     | 24.5                  |
| O77:H1    | 1               | 21.8                  | –                     | 23.1                  |
| O79:H1    | 1               | 24.5                  | –                     | 24.7                  |
| O9:K9:H12 | 5               | 18.6–23.2             | 21.6–23.1             |
| O9:H12    | 4               | 17.0–17.9             | –                     | 16.0–22.6             |
| O11:H12   | 2               | 18.2–22.4             | –                     | 21.7–22.6             |
| O20:H12   | 1               | 15.2–16.9             | 20.4–21.1             |
| O49:H12   | 1               | 19.6                  | –                     | 24.5                  |
| O55:H12   | 2               | 20.1–22.8             | 22.5–22.7             |
| O79:H12   | 1               | 22.8                  | –                     | 23.0                  |
| O98:H12   | 1               | 22.6                  | –                     | 23.4                  |
| O104:H12  | 2               | 20.9–23.6             | 22.1–23.4             |
| O118:H12  | 3               | 18.0–23.1             | 17.1–23.4             |
| O198:H12  | 4               | 16.8–23.6             | 15.9–23.2             |
| O153:H12  | 3               | 18.3–20.9             | 22.2–23.2             |
| O157:H12  | 3               | 18.7–23.4             | 21.7–23.7             |
| OONT:H12  | 2               | 21.7–22.8             | 22.5–23.4             |
| Or:H12    | 2               | 19.2–20.7             | 23.0–23.3             |

<sup>a</sup>Range of real time PCR cycle thresholds. Negative reactions are indicated with the “–” sign.
<sup>b</sup>Reference strain Orskov non-motile and the flc-genotype was detected by nucleotide sequencing of flc PCR products.
TABLE 5 | Cross-reactions of \textit{fliC}\textsubscript{H1} and \textit{fliC}\textsubscript{H12} real time PCR assays with other flagellar types of \textit{E. coli}.

| Reference strain\textsuperscript{a} | H-type | GenBank Accession No. | Detector tested\textsuperscript{b} | CT-value\textsuperscript{c} | Mismatch FP/P/RP\textsuperscript{d} |
|-------------------------------------|--------|----------------------|---------------------------------|-----------------|-----------------|
| A20 H6 AY249991.1                  | \textit{fliC}\textsubscript{H1} | 26.8                 | 0/0/0                           |                  |
| U5-41 H7 AB028474.1                | \textit{fliC}\textsubscript{H1} | 26.0                 | 0/1/0                           | 0/2/1            |
| E39a H15 AY249999.1                | \textit{fliC}\textsubscript{H1} | 28.2                 | 1/1/1                           |                  |
| H3006 H20 AY250003.1               | \textit{fliC}\textsubscript{H1} | 27.0                 | 0/0/0                           |                  |
| HW30 H28 AY337469.1                | \textit{fliC}\textsubscript{H1} | –                   | 0/2/1                           |                  |
| HW33 H31 AF345849.1                | \textit{fliC}\textsubscript{H1} | –                   | 0/2/4                           |                  |
| BP 12665 H34 AY250016.1            | \textit{fliC}\textsubscript{H1} | 20.9                 | 0/0/0                           |                  |
| P11a H37 AY250017.1                | \textit{fliC}\textsubscript{H1} | 26.4                 | 1/0/1                           |                  |
| RVC1787 H41 AY250020.1             | \textit{fliC}\textsubscript{H1} | 27.1                 | 0/1/1                           |                  |
| 4106-64 H45 AY250023.1             | \textit{fliC}\textsubscript{H1} | 25.5                 | 0/0/0                           |                  |
| S306-56 H46 AY250024.1             | \textit{fliC}\textsubscript{H1} | 27.6                 | 0/0/1                           |                  |
| 2147-59 H49 AY250026.1             | \textit{fliC}\textsubscript{H1} | 24.0                 | 0/1/1                           |                  |
| C2187-69 H52 AY250028.1            | \textit{fliC}\textsubscript{H1} | 26.8                 | 0/0/1                           |                  |
| Su1242 H1 AB028471.1               | \textit{fliC}\textsubscript{H1} | 21.1–24.3            | 0/0/0                           |                  |
| B316/42 H12 AY249997.1             | \textit{fliC}\textsubscript{H1} | 18.6–23.6            | 0/0/0                           |                  |

\textsuperscript{a} H-type reference strains (Orskov and Orskov, 1984).

\textsuperscript{b} As listed in Table 1.

\textsuperscript{c} Mean of real-time cycle threshold (CT-values) calculated from duplicate PCRs. Negative reactions are indicated with the “–” sign.

\textsuperscript{d} Number of mismatches found between real-time detector sequence and target gene sequence. FP, forward primer; P, gene probe; RP, reverse primer.

DISCUSSION

The genetically and serologically closely related flagellar antigens H1 and H12 were found in heterogeneous types of \textit{E. coli} strains belonging to 26 different O-serogroups, O-untypable and O-rough strains. With one exception (O79:H1 and O79:H12), H1 and H12 strains did not share common O-serogroups which would indicate that flagellar types H1 and H12 have separated from each other not very recently in evolution. They may have evolved independently following rearrangements in the O-group loci of ancestor strains carrying the closely related H1/H12 flagellar types and do not directly derive from a common O-group ancestor.

By comparing nucleotide sequences of \textit{flic} genes from thirteen H1 and eight H12 strains we identified six H-type specific aa changes at positions 302, 340, 361, 391, 396, and 430. All these are located in the variable part of flagellin determining antigen specificity (Wang et al., 2003). As these changes are characteristic for the respective flagellar antigen, we suppose them to determine the antigen specificities of H1 and H12. The few other aa changes detected in some H1 and H12 strains might thus not be significant as specific characteristics of H1 or H12 types. However, such aa-changes could explain the finding of serological subtypes of H1 which were detected using factor specific H-antisera (Ratiner et al., 1995).

Interestingly, the genetic distance between \textit{flic}\textsubscript{H1} (Su1242, GenBank accession AB028471.1) and \textit{flic}\textsubscript{H12} sequences (Bi 316-42, GenBank accession AY249997) (97.5% similarity) is less than that found between different alleles of \textit{flic}\textsubscript{H2a} (92.0% similarity) (Beutin et al., 2015b). It is slightly bigger than the distance found among different alleles of \textit{flic}\textsubscript{H19} (98.5% similarity) (Beutin et al., 2015a). Multiple allelic types of \textit{flic} were also detected in \textit{E. coli} H6, H7, H8, H25, and H40 strains, respectively (Reid et al., 1999; Wang et al., 2000; Beutin and Strauch, 2007; Beutin et al., 2015b). Already, a considerable number of serological cross-reactions were observed when flagellar types H1–H56 were compared (Orskov and Orskov, 1984; Edwards and Ewing, 1986). Some of these flagellar antigens (H1/H12, H8/H40, H11/H21, and H37/H41) are so closely related that the use of cross-absorbed antisera is needed to obtain unambiguous serotyping results (Edwards and Ewing, 1986).

The presence of allelic subtypes within \textit{flic} genes encoding different H-types of \textit{E. coli} and the finding that different flagellar types are serologically cross-reacting may complicate \textit{E. coli} strain typing using H-antisera. The use of molecular typing procedures, such as real-time PCR can solve the typing problem caused by serologically closely related H-antigens, as we have shown for H1 and H12 in this work. Using primer express V3.0 software, it was not possible to design real-time PCRs specific exclusively for \textit{flic}\textsubscript{H1} and \textit{flic}\textsubscript{H12}, respectively. For this reason, we employed a two-step real-time detection procedure. The first step uses a real-time PCR highly specific for both H1 and H12
TABLE 6 | Reaction of the fliC<sub>H1/H12</sub>, fliC<sub>H1</sub> and fliC<sub>H12</sub> real-time PCR assays with non-H1 and non-H12 strains.

| Serotype  | Number of strains | Ct-values<sup>a</sup> fliC<sub>H1/H12</sub> | Ct-values<sup>a</sup> fliC<sub>H1</sub> | Ct-value<sup>a</sup> fliC<sub>H12</sub> |
|-----------|-------------------|---------------------------------|---------------------------------|---------------------------------|
| O33:H6    | 1                 | –                               | 20.6                            | –                               |
| O40:H6    | 1                 | –                               | 19.35                           | –                               |
| O55:H6    | 1                 | –                               | 17.2                            | –                               |
| O63:H6    | 1                 | –                               | 18.98                           | –                               |
| O113:H6   | 2                 | –                               | 19.38–19.46                     | –                               |
| O125:H6   | 1                 | –                               | 24.29                           | –                               |
| O126:H6   | 1                 | –                               | 20.17                           | –                               |
| O127:H6   | 1                 | –                               | 21.78                           | –                               |
| O41:H7    | 1                 | –                               | –                               | 21.11                           |
| O55:H7    | 3                 | –                               | –                               | 22.35–26.16                     |
| O153:H7   | 1                 | –                               | –                               | 18.21                           |
| O157:H7   | 4                 | –                               | –                               | 18.46–24.7                      |
| O23:H15   | 1                 | –                               | –                               | –                               |
| O157:H15  | 1                 | –                               | –                               | –                               |
| O28:H28   | 1                 | –                               | –                               | 18.73                           |
| O91:H28   | 1                 | –                               | –                               | 16.34                           |
| O110:H28  | 1                 | –                               | –                               | 15.53                           |
| O116:H28  | 1                 | –                               | –                               | 30.55                           |
| O145:H28  | 1                 | –                               | –                               | 14.99                           |
| OX185:H28 | 1                 | –                               | –                               | 17.18                           |
| O51:H49   | 1                 | –                               | –                               | 21.1                            |
| O114:H49  | 1                 | –                               | –                               | 20.65                           |
| O181:H49  | 1                 | –                               | –                               | 20.6                            |
| O45:H31   | 1                 | –                               | –                               | 19.44                           |
| O179:H31  | 1                 | –                               | –                               | 19.56                           |
| O6:H34    | 2                 | –                               | –                               | –                               |
| O86:H34   | 1                 | –                               | –                               | 21.4                            |
| O142:H34  | 1                 | –                               | –                               | 21.99                           |
| O415:H34  | 1                 | –                               | –                               | 21.67                           |
| O312:H34  | 1                 | –                               | –                               | –                               |
| O312:H34  | 1                 | –                               | –                               | 20.84                           |
| O76:H41   | 1                 | –                               | –                               | 20.75                           |
| O1777:H41 | 1                 | –                               | –                               | 21.93                           |
| O8:H45    | 1                 | –                               | –                               | –                               |
| O121:H45  | 1                 | –                               | –                               | 22.32                           |
| O157:H45  | 1                 | –                               | –                               | 24.34                           |
| O186:H45  | 1                 | –                               | –                               | 20.92                           |
| O119[H52] | 1                 | –                               | –                               | –                               |
| O2:H8     | 1                 | –                               | –                               | –                               |
| O2:H25    | 1                 | –                               | –                               | 27.15                           |
| O2:H27    | 1                 | –                               | –                               | –                               |
| O2:H40    | 1                 | –                               | –                               | –                               |
| O4:H5     | 1                 | –                               | –                               | –                               |
| O4:H16    | 1                 | –                               | –                               | –                               |
| O6        | 2                 | –                               | –                               | –                               |
| O6:H4     | 1                 | –                               | –                               | 24.17                           |
| O6:H10    | 1                 | –                               | –                               | –                               |
| O7:H4     | 1                 | –                               | –                               | –                               |
| O15:H2    | 1                 | –                               | –                               | –                               |

<sup>a</sup>Range of real time PCR cycle thresholds. Negative reactions are indicated with the “–” sign.

(Continued)

TABLE 6 | Continued

| Serotype  | Number of strains | Ct-values<sup>a</sup> fliC<sub>H1/H12</sub> | Ct-values<sup>a</sup> fliC<sub>H1</sub> | Ct-value<sup>a</sup> fliC<sub>H12</sub> |
|-----------|-------------------|---------------------------------|---------------------------------|---------------------------------|
| O15:H11   | 1                 | –                               | –                               | –                               |
| O15:H16   | 1                 | –                               | –                               | –                               |
| O15:K21   | 1                 | –                               | –                               | –                               |
| O139:H4   | 1                 | –                               | –                               | –                               |
| O139:H19  | 1                 | –                               | –                               | –                               |
| O128:H2   | 1                 | –                               | –                               | –                               |
| O128:H8   | 1                 | –                               | –                               | –                               |
| O20:H9    | 1                 | –                               | –                               | 23.23                           |
| O20:H30   | 1                 | –                               | –                               | –                               |
| O20:H33   | 1                 | –                               | –                               | –                               |
| O20:NM    | 1                 | –                               | –                               | –                               |
| O55:H19   | 1                 | –                               | –                               | 23.43                           |
| O55:H21   | 1                 | –                               | –                               | –                               |
| O55:H51   | 1                 | –                               | –                               | –                               |
| O118:H2   | 1                 | –                               | –                               | –                               |
| O118:H5   | 1                 | –                               | –                               | –                               |
| O118:H8   | 1                 | –                               | –                               | –                               |
| O118:H16  | 1                 | –                               | –                               | –                               |
| O153:H14  | 1                 | –                               | –                               | 20.86                           |
| O153:H21  | 1                 | –                               | –                               | –                               |
| O153:H25  | 1                 | –                               | –                               | 28.37                           |

Strains, followed by subtyping of H1/H12-positive strains with the respective fliC<sub>H1</sub> and fliC<sub>H12</sub> specific real-time PCRs. Short probe sequence lengths as obtained with minor groove binder (MGB) or MGB-replacements (XS-probe) are needed to ensure specificity between closely similar DNA-targets as previously shown for fliC<sub>H19</sub> allelic discrimination (Beutin et al., 2015a). The PCRs could be used in parallel for examination of large number of isolates using high throughput PCR platforms as described previously for analysis of large numbers of Clostridia and E. coli strains (Delannoy et al., 2013; Woudstra et al., 2013).

Unambiguous typing of fliC<sub>H1</sub> and fliC<sub>H12</sub> sequences is of interest for clinical and epidemiological investigations since some H1 and H12 strains were shown to play a role as pathogens in humans and animals.

More than one third of investigated H1 and H12 strains produced Shiga toxins. Strains showing O:H types characteristic for ExPEC associated with human diseases (O2:H1, O4:H1, O6:H1, O15:H1) were detected in this work. Interestingly, these were not only from humans but also found in animals and food. It was previously described that animals, food and water can be a source of pandemic ExPEC strains (Jakobsen et al., 2010; Riley, 2014; Gomi et al., 2015; Singer, 2015). Flagellar type H12 strains encompass mainly STEC (O20:H12, O55:H12, O118:H12, O136:H12, O153:H12, and Or:H12) and were isolated from diseased animals and humans, food and the environment (Scheutz and Stockbaine, 2005).
The specific molecular detection of H1 and H12 flagellins as described in this study will be useful for diagnosis and for source attribution of human and animal pathogenic ExPEC and STEC strains in outbreaks and sporadic cases of infection.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: LB, SD, PF. Performed the experiments: LB, SD, PF. Analyzed the data: LB, SD, PF. Contributed reagents/materials/analysis tools: LB, SD, PF. Wrote the paper: LB, SD, PF. Critical revision of the paper for important intellectual content: LB, SD, PF.

REFERENCES

Allen, C. A., Niesel, D. W., and Torres, A. G. (2008). The effects of low-shear stress on Adherent-invasive Escherichia coli. Environ. Microbiol. 10, 1512–1525. doi: 10.1111/j.1462-2920.2008.01567.x

Bettelheim, K. A. (1978). The sources of “OH” serotypes of Escherichia coli. J. Hyg. 80, 83–113. doi: 10.1017/S0022283600053420

Beutin, L., Delannoy, S., and Fach, P. (2015a). Genetic diversity of the fliCH19 genes encoding the flagellar antigen H19 of Escherichia coli and application to the specific identification of enterohemorrhagic E. coli (EHEC) O121:H19. Appl. Environ. Microbiol. 81, 4224–4230. doi: 10.1128/AEM.01264-15

Beutin, L., Delannoy, S., and Fach, P. (2015b). Sequence variations in the flagellar antigen genes fliCH25 and fliCH28 of Escherichia coli and their use in identification and characterization of enterohemorrhagic E. coli (EHEC) O145:H25 and O145:H28. PLoS ONE 10:e0126749. doi: 10.1371/journal.pone.0126749

Beutin, L., Fach, P., and F. (2014). “Detection of Shiga toxin-producing Escherichia coli from nonhuman sources and strain typing” in Microbiology Spectrum, eds V. Sperandio and C. J. Hovde (Washington, DC: American Society for Microbiology), 1–23.

Beutin, L., Krause, G., Zimmermann, S., Kaufuss, S., and Gleier, K. (2004). Characterization of Shiga toxin-producing Escherichia coli strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. 42, 1099–1108. doi: 10.1128/JCM.42.6.1099-1108.2004

Beutin, L., Kruger, U., Krause, G., Mikó, A., Martin, A., and Strauch, E. (2008). Evaluation of major types of Shiga toxin 2e producing Escherichia coli present in food, pigs and in the environment as potential pathogens for humans. Appl. Environ. Microbiol. 74, 4806–4816. doi: 10.1128/AEM.00623-08

Beutin, L., and Strauch, E. (2007). Identification of sequence diversity in the Escherichia coli fliC genes encoding flagellar types H8 and H40 and its use in typing of Shiga toxin-producing E. coli O1, O22, O111, O117, and O179 strains. J. Clin. Microbiol. 45, 333–339. doi: 10.1128/JCM.01627-06

Bugarel, M., Beutin, L., Martin, A., Gill, A., and Fach, P. (2010). Microarray for the identification of Shiga toxin-producing Escherichia coli (STEC) seropathotypes associated with Hemorrhagic Colitis and Hemolytic Uremic Syndrome in humans. Int. J. Food Microbiol. 142, 318–329. doi: 10.1016/j.ijfoodmicro.2010.07.010

Clotilde, I. M., Salvador, A., Bernard, C., Lin, A., Lauzon, C., Muldoon, M., et al. (2015). Comparison of multiplex immunochemical and molecular serotyping methods for shiga toxin-producing Escherichia coli. Foodborne Pathog. Dis. 12, 118–121. doi: 10.1089/fpd.2014.1827

Delannoy, S., Beutin, L., and Fach, P. (2013). Discrimination of enterohemorrhagic Escherichia coli (EHEC) from non-EHEC strains based on detection of various combinations of type III effector genes. J. Clin. Microbiol. 51, 3257–3262. doi: 10.1128/JCM.01471-13

Eaves-Pyles, T., Allen, C. A., Taormina, J., Swidsinski, A., Tutt, C. B., Jezek, G. E., et al. (2008). Escherichia coli isolated from a Crohn’s disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. Int. J. Med. Microbiol. 298, 397–409. doi: 10.1016/j.ijmm.2007.05.011

Echeverria, P., Orskov, F., Orskov, I., and Planbangchang, D. (1982). Serotypes of enterotoxigenic Escherichia coli in Thailand and the Philippines. Infect. Immun. 36, 851–856.

Edwards, P. R., and Ewing, W. H. (1986). “The genus Escherichia,” in Identification of Enterobacteriaceae. 4th Edn., ed W. H. Ewing (New York, NY: Elsevier Science Publishing Co.), 93–136.

Fairbrother, J. M., Nadeau, E., and Gyles, C. L. (2005). Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6, 17–39. doi: 10.1079/AHR2005105

Fratamico, P. M., Bagi, L. K., Cray, W. C. Jr., Narang, N., Yan, X., Medina, M., et al. (2011). Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing Escherichia coli serogroups O26, O45, O103, O111, O121, and O145 in ground beef. Foodborne Pathog. Dis. 8, 601–607. doi: 10.1089/fpd.2010.0773

Frynddelahl, K. (2002). Prevalence of serogroups and virulence genes in Escherichia coli associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Vet. Microbiol. 85, 169–182. doi: 10.1016/S0378-1135(01)00504-1

Geser, N., Stephan, R., Korczak, B. M., Beutin, L., and Hachler, H. (2012). Molecular identification of extended-spectrum-beta-lactamase genes from enterobacteriaceae isolated from healthy human carriers in Switzerland. Antimicrob. Agents Chemother. 56, 1609–1612. doi: 10.1128/AAC.05539-11

Gomi, R., Matsuda, T., Fujimori, Y., Harada, H., Matsu, Y., and Yoneda, M. (2015). Characterization of pathogenic Escherichia coli in river water by simultaneous detection and sequencing of 14 virulence genes. Environ. Sci. Technol. 49, 6800–6807. doi: 10.1021/acs.est.5b00953

Goulter, R. M., Gentle, I. R., and Dykes, G. A. (2010). Characterisation of curli production, cell surface hydrophobicity, autoaggregation and attachment behaviour of Escherichia coli O157. Curr. Microbiol. 61, 157–162. doi: 10.1007/s00284-010-9589-2

Hampton, D. J., Fu, Z. F., Bettelheim, K. A., and Wilson, M. W. (1988). Manometrical influences on the selective proliferation of two strains of haemolytic Escherichia coli in weaned pigs. Epidemiol. Infect. 100, 213–220. doi: 10.1017/S0950268800067340

Iguchi, A., Iyoda, S., Kikuchi, T., Ogura, Y., Katsura, K., Ohnishi, M., et al. (2015a). A complete view of the genetic diversity of the Escherichia coli O-antigen biosynthesis gene cluster. DNA Res. 22, 101–107. doi: 10.1093/dnares/dsu043

Iguchi, A., Iyoda, S., Seto, K., Morita-Ishihara, T., Scheutz, F., and Ohnishi, M. (2015b). Escherichia coli O-Genotyping PCR: a Comprehensive and practical platform for molecular O Serogrouping. J. Clin. Microbiol. 53, 2427–2432. doi: 10.1128/JCM.00321-15

Jakobsen, L., Spangholm, D. J., Pedersen, K., Jensen, L. B., Emborg, H. D., Agero, Y., et al. (2010). Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in Escherichia coli isolates from community-dwelling humans and UTI patients. Int. J. Food Microbiol. 142, 264–272. doi: 10.1016/j.ijfoodmicro.2010.06.025

Joensen, K. G., Tetzchner, A. M., Iguchi, A., Arestrup, F. M., and Scheutz, F. (2015). Rapid and easy in silico serotyping of Escherichia coli isolates by...

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use of whole-genome sequencing data. J. Clin. Microbiol. 53, 2410–2426. doi: 10.1128/JCM.00088-15

Johnson, J. R., Orskov, I., Orskov, F., Goullet, P., Picard, B., Moseley, S. L., et al. (1994). O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among Escherichia coli strains causing urosepsis. J. Infect. Dis. 169, 119–126. doi: 10.1093/infdis/169.1.119

Johnson, J. R., Owens, K., Gajewski, A., and Kuskowski, M. A. (2005). Bacterial characteristics in relation to clinical source of Escherichia coli isolates from women with acute cystitis or pylonephritis and uninfected women. J. Clin. Microbiol. 43, 6064–6072. doi: 10.1128/JCM.43.12.6064-6072.2005

Johnson, J. R., Owens, K. L., Clabots, C. R., Weissman, S. J., and Cannon, S. B. (2006). Phylogenetic relationships among clonal groups of extraintestinal pathogenic Escherichia coli as assessed by multi-locus sequence analysis. Microbes Infect. 8, 1702–1713. doi: 10.1016/j.micinf.2006.02.007

Kaper, J. B., Nataro, J. P., and Mobley, H. L. (2004). Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2, 123–140. doi: 10.1038/nrmicro818

Kaufmann, M., Zweifel, C., Blanco, M., Blanco, J. E., Blanco, J., Beutin, L., et al. (2009). Biofilm formation as a novel phenotypic feature of adherent- toxin-producing E. coli isolates from uropathogenic Escherichia coli flagellin (fliC) alleles in Pathogenic Escherichia coli. J. Bacteriol. 181, 153–160. doi: 10.1128/JCM.00008-15

Reister, M., Hoffmeier, K., Kreizdorn, N., Rotter, B., Liang, C., Biro, S., et al. (2014). Complete genome sequence of the gram-negative probiotic Escherichia coli strain Nissle 1917. J. Biotechnol. 187, 106–107. doi: 10.1016/j.jbiotec.2014.07.042

Riley, L. W. (2014). Pandemic lineages of extraintestinal pathogenic Escherichia coli. Clin. Microbiol. Infect. 20, 380–390. doi: 10.1111/1469-0691.12646

Scheutz, F., Cheasty, T., Woodward, D., and Smith, H. R. (2004). Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new E. coli O groups that include Verocytotoxin-producing E. coli (VTEC): O176, O177, O178, O179, O180 and O181. APMIS 112, 569–584. doi: 10.1111/j.1600-0463.2004.apm10209.0

Scheutz, F., and Stockbne, N. A. (2005). “Genus I. Escherichia,” in Bergey’s Manual of Systematic Bacteriology, 2nd Edn., eds G. M. Garrity, D. J. Brenner, N. R. Krieg, and J. T. Staley (New York, NY: Springer), 667–674.

Schultz, F., Teel, L. D., Beutin, L., Pierard, D., Buvens, G., Karch, H., et al. (2012). Multicenter evaluation of a sequence-based protocol for subtyping shiga toxins and standardizing nomenclature. J. Clin. Microbiol. 50, 2951–2963. doi: 10.1128/JCM.00086-12

Shaheen, H. I., Khalil, S. B., Rao, M. R., Abu, E. R., Wierzbz, T. F., Peruski, L. F. Jr., et al. (2004). Phenotypic profiles of enterotoxigenic Escherichia coli associated with early childhood diarrhea in rural Egypt. J. Clin. Microbiol. 42, 5588–5595. doi: 10.1128/JCM.42.12.5588-5595.2004

Singer, R. S. (2015). Urinary tract infections attributed to diverse ExPEC strains in food animals: evidence and data gaps. Front. Microbiol. 6. 628. doi: 10.3389/fmicb.2015.00628

Stenutz, R., Weintraub, A., and Widmalm, G. (2006). The structures of Escherichia coli O-poly saccharide antigens. FEMS Microbiol. Rev. 30, 382–403. doi: 10.1111/j.1574-6976.2006.00016.x

Tajima, F., and Nei, M. (1984). Estimation of evolutionary distance between nucleotide sequences. Mol. Biol. Evol. 1, 269–285.

Tschepe, H., Bender, L., Ott, M., Wittig, W., and Hacker, J. (1992). Restriction fragment length polymorphism and virulence pattern of the veterinary pathogen Escherichia coli O139:K82:H1. Zentralbl. Bakteriol. 276, 264–272. doi: 10.1007/s003130050029

Wang, L., Rothemund, D., Kurd, H., and Reeves, P. R. (2000). Sequence diversity of the Escherichia coli H7 fliC genes: implication for a DNA-based typing scheme for E. coli O157:H7. J. Clin. Microbiol. 38, 1786–1790.

Wang, L., Rothemund, D., Kurd, H., and Reeves, P. R. (2003). Species-wide variation in the Escherichia coli flagellin (H-Antigen) gene. J. Bacteriol. 185, 2936–2943. doi: 10.1128/JB.185.9.2936-2943.2003

Wang, Q., Wang, S., Beutin, L., Cao, B., Feng, L., and Wang, L. (2010). Development of a DNA microarray for detection and serotyping of enterotoxigenic Escherichia coli. J. Clin. Microbiol. 48, 2066–2074. doi: 10.1128/JCM.02341-09

Welch, R. A., Burland, V., Blunkett, G. III, Redford, P., Roesch, P., Rasko, D., et al. (2002). Extensive mosaic structure revealed by the complete genome sequence of uropathogenic Escherichia coli. Proc. Natl. Acad. Sci. U.S.A. 99, 17020–17024. doi: 10.1073/pnas.252529799

Woudstra, C., Lambert, D., Anniballi, F., De Medicis, D., Austin, J., and Fach, P. (2013). Genetic diversity of the flagellin genes of Clostridium botulinum groups I and II. Appl. Environ. Microbiol. 79, 3926–3932. doi: 10.1128/AEM.00868-13

Zdziarski, J., Brusziszewicz, E., Wullt, B., Liesegang, H., Biran, D., Voigt, B., et al. (2010). Host imprint on bacterial genomes—rapid, divergent evolution in individual patients. PLoS Pathog. 6:e1001078. doi: 10.1371/journal.ppat.1001078

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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