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Novel Microarray Design for Molecular Serotyping of Shiga Toxin-Producing *Escherichia coli* Strains Isolated from Fresh Produce

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Serotyping *Escherichia coli* is a cumbersome and complex procedure due to the existence of large numbers of O- and H-antigen types. It can also be unreliable, as many Shiga toxin-producing *E. coli* (STEC) strains isolated from fresh produce cannot be typed by serology or have only partial serotypes. The FDA *E. coli* identification (FDA-ECID) microarray, designed for characterizing pathogenic *E. coli*, contains a molecular serotyping component, which was evaluated here for its efficacy. Analysis of a panel of 75 reference *E. coli* strains showed that the array correctly identified the O and H types in 97% and 98% of the strains, respectively. Comparative analysis of 73 produce STEC strains showed that serology and the array identified 37% and 50% of the O types, respectively, and that the array was able to identify 16 strains that could not be O serotyped. Furthermore, the array identified the H types of 97% of the produce STEC strains compared to 65% by serology, including six strains that were mistyped by serology. These results show that the array is an effective alternative to serology in serotyping environmental *E. coli* isolates.

Pathogenic *Escherichia coli* bacteria are classified based on the virulence factors they carry or the clinical manifestations they cause, so serotyping is not very useful for identifying these pathogens in general. One exception may be Shiga toxin-producing *E. coli* (STEC) of serotype O157:H7, which is recognized worldwide by its somatic (O) 157 and flagellar (H) 7 antigens. Still, serological typing continues to provide important metadata that are critical in epidemiological trace-backs, outbreak investigations, and surveillance programs. Also, as selected non-O157 STEC strains are being implicated more often in food-borne infections worldwide, the need to serotype STEC isolates has also increased in importance.

E. coli can theoretically carry any combination of the known 181 O and 53 H antigens (1), so serotyping is a labor-intensive resource that often only reference laboratories have the capacity to perform. Traditional *E. coli* serotyping, which takes a few weeks to complete, uses multiple pools of polyclonal antisera to the known O and H antigens to screen an isolate. The pool that reacts with the isolate is then retested using the individual antisera that comprised the pool to determine the specific O and H types. Aside from the costs and difficulties of maintaining O- and H-antiserum panels, new batches of antisera made to replenish depleted reagents may exhibit slight variations in specificity, avidity, or cross-reactivity, which can affect consistency and reproducibility. Also, due to the large numbers of *E. coli* serotypes that exist, it is not unusual to find strains that cannot be serotyped, so the effectiveness of serotyping can be limited. For example, studies of STEC and enterotoxigenic *E. coli* (ETEC) strains isolated from fresh produce found that over 50% of the isolates could not be typed or yielded only partial serotypes (2, 3).

As an alternative to using antisera, *E. coli* strains can be molecularly serotyped by testing for the presence of specific antigenic gene sequences. Genetic assays are especially useful for serotyping strains that are not well characterized or where antisera are not available. For instance, a genetic H-serotyping method using the *fliC* gene that encodes the flagellar structural subunit (4) was used to determine the H type of some STEC strains isolated from fresh produce (5). Other investigators have developed broader genetic H serotyping by using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis, where *fliC* gene amplicons are restricted and fractionated on agarose gels to look for H-type-specific banding patterns (6). A similar PCR-RFLP assay was developed to identify 147 *E. coli* O serogroups (7), but these RFLP assays are fairly labor-intensive. As an alternative, DNA microarrays are an efficient platform to simultaneously analyze a large number of gene targets. One microarray assay identified 15 *Shigella* and *E. coli* serotypes, including five pathogenic *E. coli* O types associated with disease (8). Another array could serotype 24 epidemiologically relevant *E. coli* O types and included 47 H types (9), but it was not broadly applicable to other O types.

A custom Affymetrix DNA microarray was developed by the FDA for the identification and characterization of pathogenic *E. coli* strains. The array, designated FDA *E. coli* identification (FDA-ECID), incorporates genetic signatures from over 250 whole-genome sequences, more than 40,000 *E. coli* genes, and approximately 9,800 single nucleotide polymorphisms to provide a nearly true representation of the *E. coli* pangenome. A molecular serotyping component was also included in the design, in which 147 *E. coli* O antigens are represented by 103 *wzx*, 103 *wzy*, and 5 *wzm* alleles that are involved in O-antigen biosynthesis (10). In addition, 53 H antigens are represented on the array by probes targeting the allele-specific regions of the known flagellin genes (*fliC*, *flkA*, *fllA*, *flmA*, and *flnA*) (11). Since *E. coli* strains are diverse and since environmental isolates are difficult to serotype, we examined

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the potential of using the FDA-ECID array to serotype *E. coli* strains, first by using a panel of reference strains and then by using STEC strains that were isolated from fresh produce and that could not be fully typed by serology.

MATERIALS AND METHODS

Bacterial strains. To establish the effectiveness of the microarray to serotype *E. coli* isolates, we used strains from the diarrheagenic *E. coli* (DEC) reference collection obtained from the STEC Center (http://www.shigatox.net/stec/cgi-bin/deca) at Michigan State University. The DEC collection is comprised of well-characterized strains of 21 different serotypes that have been isolated worldwide and is representative of the 15 common diarrheagenic *E. coli* clones. All 73 environmental STEC strains were isolated from fresh produce (2). These consisted of 11 strains that were not serotyped and 62 strains that were typed by the *E. coli* Reference Center at Pennsylvania State University using O antisera and H typing by PCR-RFLP of the *fliC* gene (6). Of these, nine strains were fully serotyped, but the rest had either partial or no serotype data. The microarray analyses of the produce STEC strains were done as a blind study, and the serotype of each strain determined by the array was compared to the known serological data.

Microarray assay. Pure isolates of each strain were grown at 37°C overnight in 3 ml of Luria broth. On the next day, total DNA from 1 ml of culture was extracted and isolated using a DNeasy kit (Qiagen, Valencia, CA) and digested with RQ1 RNase-Free DNase I (Amersham Biosciences Corp., now GE Life Sciences, Piscataway, NJ). The 20-μl digestion reaction mixtures contained 2 μg of DNA, 1× One-Phor-All-Buffer (GE Life Sciences), and 0.01 U of DNase I enzyme. The digested DNA, which had an average fragment size of ~200 bp, was then 3′ labeled with biotin using 30 U of terminal deoxynucleotidyl transferase (rTdT; Promega Corp., Madison, WI) and biotin-11-ddATP (PerkinElmer, Akron, OH) in 1× terminal transferase buffer. The labeling reaction was done for a minimum of 3 h. Biotinylated DNA was hybridized to the FDA-ECID microarray for 16 h at 45°C using the buffer provided by a 3′ IVT Express kit (Affymetrix). Posthybridization washing, staining, and scanning were performed on the Affymetrix GeneAtlas instrument also using the 3′ IVT Express kit according to the manufacturer's recommendations.

Microarray design. Gene sequences that encode the various O and H antigens were mined from various public databases, such as NCBI. All of these sequences were then sent to Affymetrix, where the actual probe design was performed and where the arrays were manufactured. In order to discriminate between closely related alleles of the same gene, each gene/allele target is represented on the FDA-ECID array by 22 individual oligonucleotide (25-mer) probes. Collectively, these 22 probes are referred to as a probe set, and within a probe set, there are 11 probe pairs. A probe pair consists of a perfect match (PM) probe and a mismatch (MM) probe. As the name suggests, the PM probe matches the target/reference sequence perfectly while the MM probe contains a single nucleotide mismatch in the central (13th) position. MM probe sequences were generated using Affymetrix's standard homomeric substitution routine. The rationale for including the MM probe is to allow measurement of, and thereby correction for, nonspecific hybridization. Individual PM probe sequences are available in the supplemental material.

Microarray data analysis. Gene targets are determined to be either present or absent by a *P* value calculated from the discrimination score (*R*) for each probe pair as described previously (12). Briefly, *R* is a basic property of a probe pair that describes its ability to detect its intended target. It measures the target-specific intensity difference of the probe pair (PM − MM) relative to its overall hybridization intensity (PM + MM) and is defined as (PM − MM)/(PM + MM). Next, each of the 11 *R* values is compared to the user-definable threshold τ, a small positive number that can be adjusted to increase or decrease sensitivity and/or specificity of the analysis. A one-sided Wilcoxon signed-rank test is then employed to generate the detection *P* value. During this test, each probe pair is assigned a rank based on how far its discrimination score is from τ. Probe sets with detection *P* values of <0.05 are scored as "present." The MAS5 Gene Calls analysis routine was run in R-Bioconductor using the affy package mas5calls function with the user-defined parameters $\tau = 0.15$, $\alpha1 = 0.05$, and $\alpha2 = 0.05$. The *P* values calculated for each molecular serotyping probe set are available in the supplemental material.

RESULTS

DEC strains. Results of microarray analysis for O typing of the DEC strains are shown in Table 1. The panel consisted of 75 DEC strains from seven O serogroups. Overall, 97% (73/75) of the strains tested by the array gave O-type identifications that were consistent with the known O types of these strains. Ten of the DEC strains were previously determined to be nonmotile, but the array was able to determine the molecular H types of these strains. With the exception of strains DEC 7b and DEC 14a, the H types obtained were consistent with the observed H serotypes of the other members of the same DEC clone (Table 1). Since the nonmotile strains had no known H serotype, they were not included in the comparison of the two serotyping methodologies. Overall, the array agreed with H serology in 64/65 strains, for a 98% H-type consistency rate. Strains DEC 7b and DEC 14a gave discrepant results between the two methodologies. DEC 7b is reported to be O149:H− but was typed by the array as O157:H42, while DEC 14a is reported to be O128:H21 but was typed as O86:H8. Analysis of the genetic sequences of these strains were found to concur with the molecular serotypes identified by the array, so both strains appear to have been mislabeled or were mistyped by serology.

Produce STEC strains. Results of array serotyping of produce STEC strains are summarized in Table 2. Excluding the 11 strains for which traditional serotyping was not performed, serology identified the O type of 23/62 (37%) STEC strains, including four strains that had the OX classification. Of these, the array correctly matched the O type of 13/23 strains, and the O73 strain 2364 was determined by the array to be within the O17/O44/O73/O77/O106 group, members of which share close similarities in antigenic structure. Of the four strains classified as OX by serology, three were not identified by the array, but strain 2379 was found to have the antigen sequences of *Shigella boydii* type 9 (B9). The other five strains that were not identified by the array but had an O type by serology were strains 2344 (O88), 2346 (O107), 2351 (O1 weak reaction), 2353 (O1), and 2357 (O76) (Table 2). In contrast, there were 16 STEC strains that could not be O serotyped but for which the array was able to identify an O type: B9 (six strains), O130 (three strains), O17/O44/O73/O77/O106 (three strains), and one strain each of O8, O22, O28ac/O42, and O45 (Table 2). Overall, the array was able to identify the O type or provide some information on the O-serogroup identity of 31/62 (50%) of the STEC strains. Lastly, there were 23 STEC strains that could not be O typed by serology or microarray.

Serotyping identified the H types of 46/62 STEC strains, and of these, the array identified the same H types in 39 strains, resulting in an 85% concurrence with serology. The seven strains that were discrepant were the following (H serotype/array H type): 2330 (H38/25), 2331 (H31/nontypeable), 2336 (H38/2), 2354 (H16/2), 2355 (H1/20), 2391 (H36/21), and 2392 (H36/21). To resolve these discrepancies, all seven strains were analyzed by *fliC* DNA sequencing. For six of the strains, the *fliC* sequences matched the H types identified by the array (data not shown), indicating that these strains were mistyped by serology. The H type of the seventh strain (2331) could not

TABLE 1 Microarray analysis of the DEC collection

DEC clone no. and strain	Reported serotype	FDA-ECID serotype[a]
1		
1a	O55:H6	O55:H6
1b	O55:H6	O55:H6
1c	O55:H6	O55:H6
1d	O55:H6	O55:H6
1e	O55:H6	O55:H6
2		
2a	O55:H6	O55:H6
2b	O55:H−	O55:H6
2c	O55:H6	O55:H6
2d	O55:H6	O55:H6
2e	O55:H6	O55:H6
3		
3a	O157:H7	O157:H7
3b	O157:H7	O157:H7
3c	O157:H7	O157:H7
3d	O157:H7	O157:H7
3e	O157:H7	O157:H7
4		
4a	O157:H7	O157:H7
4b	O157:H7	O157:H7
4c	O157:H7	O157:H7
4d	O157:H7	O157:H7
4e	O157:H7	O157:H7
5		
5a	O55:H7	O55:H7
5b	O55:H7	O55:H7
5c	O55:H7	O55:H7
5d	O55:H7	O55:H7
5e	O55:H7	O55:H7
6		
6a	O111:H12	O111:H12
6b	O111:H12	O111:H12
6c	O111:H12	O111:H12
6d	O111:H4	O111:H4
6e	O111:H4	O111:H4
7		
7a	O157:H43	O157:H43
7b	O149:H−	**O157:H42**
7c	O157:H43	O157:H43
7d	O157:H43	O157:H43
7e	O157:H−	O157:H43
8		
8a	O111:H−	O111:H8
8b	O111:H8	O111:H8
8c	O111:H−	O111:H11
8d	O111:H11	O111:H11
8e	O111:H8	O111:H8
9		
9a	O26:H11	O26:H11
9b	O26:H−	O26:H11
9c	O26:H−	O26:H11
9d	O26:H11	O26:H11
9e	O26:H11	O26:H11
10		
10a	O26:H11	O26:H11
10b	O26:H11	O26:H11
10c	O26:H11	O26:H11
10d	O26:H11	O26:H11
10e	O26:H11	O26:H11
11		
11a	O128:H2	O128:H2
11b	O128:H2	O128:H2
11c	O45:H2	O45:H2
11d	O128:H2	O128:H2
11e	O128:H2	O128:H2
12		
12a	O111:H2	O111:H2
12b	O111:H2	O111:H2
12c	O111:H−	O111:H2
12d	O111:H2	O111:H2
12e	O111:H−	O111:H2
13		
13a	O128:H7	O128:H7
13b	O128:H7	O128:H7
13c	O128:H7	O128:H7
13d	O128:H7	O128:H7
13e	O128:H7	O128:H7
14		
14a	O128:H21	**O86:H8**
14b	O128:H21	O128:H21
14c	O128:H21	O128:H21
14d	O128:H−	O128:H21
14e	O128:H21	O128:H21
15		
15a	O111:H21	O111:H21
15b	O111:H21	O111:H21
15c	O111:H21	O111:H21
15d	O111:H21	O111:H21
15e	O111:H21	O111:H21

[a] Observed inconsistencies with reported serotypes are in boldface.

be identified by the array, but sequence analysis showed that its *fliC* sequence had homologies to *Enterobacter cloacae*. Taking into account these six mistyped strains, serology actually identified the H type of only 40/62 (65%) strains, while the array identified the H types of 60/62 (97%) strains, including 15 strains that showed no H type by serology. Three strains could not be H typed by the array, and they were strain 2331 (mentioned above) and strains 2325 and 2390, all of which had *fliC* DNA sequence homologies to *E. cloacae*. To verify their identities, all three strains were tested with a Vitek 2 GN kit (bioMérieux, Hazelwood, MO) for their biochemical phenotypes and were determined to be *Enterobacter* sp. isolates (data not shown). Lastly, there were 11 strains that had not been serotyped (listed as ND in Table 2), and the array was able to identify the O and H types in 3 and 10 of these strains, respectively (Table 2).

TABLE 2 Results of serology versus microarray in serotyping produce STEC strains

Strain no.	ECRC serotype[a]	FDA-ECID serotype[b]
2322	O−:H52	ONT:H52
2323	O−:H52	ONT:H52
2324	O53:H−	O53:H20
2325	O−:H−	ONT:HNT
2326	O−:H−	O22:H8
2327	O121:H19	O121:H19
2328	O−:H52	ONT:H52
2329	O−:H52	ONT:H52
2330	O−:H38	O28ac/O42:H25
2331	O−:H31	ONT:HNT
2332	O−:H2/H35	ONT:H2
2333	O−:H8	ONT:H8
2334	O−:H16	ONT:H16
2335	O−:H16	O45:H16
2336	O−:H38	OB9:H2
2337	OX25:H−	ONT:H11
2338	O113:H21	O113:H21
2339	O8:H−	O8:H28
2340	O−:H19	ONT:H19
2341	O−:H21	ONT:H21
2342	O113:H21	O113:H21
2343	O168:H−	O168:H8
2344	O88:H−	ONT:H25
2345	O−:H49	ONT:H49
2346	O107:H−	ONT:H38
2347	O−:H2	OB9:H2
2348	O−:H28	O8:H28
2349	O−:H16	ONT:H16
2350	O−:H2	ONT:H2
2351	O1:H−	ONT:H20
2352	O−:H11	O130:H11
2353	O1:H−	ONT:H20
2354	O−:H16	OB9:H2
2355	O−:H1	ONT:H20
2356	O−:H2	OB9:H2
2357	O76:H−	ONT:H21
2358	O−:H2	ONT:H2
2359	O−:H11	O130:H11
2360	O−:H28	ONT:H28
2361	O168:H8	O168:H8
2362	O−:H30	ONT:H30
2363	O−:H49	ONT:H49
2364	O73:H−	O17/O44/O73/O77/O106:H45
2365	OX25:H11	ONT:H11
2366	O26:H11	O26:H11
2367	O−:H−	O17/O44/O73/O77/O106:H45
2368	O−:H−	O17/O44/O73/O77/O106:H45
2369	O−:H−	O17/O44/O73/O77/O106:H45
2370	O−:H7	ONT:H7
2371	O−:H7	ONT:H7
2372	O174:H21	O174:H21
2373	O21:H−	O21:H21
2374	O165:H25	O165:H25
2375	ND	ONT:H8
2376	O−:H11	O130:H11
2377	OX18:H19	ONT:H19
2378	O−:H2	OB9:H2
2379	OX23:H2	OB9:H2
2380	O−:H2	OB9:H2
2381	O−:H7	ONT:H7
2382	ND	ONT:H16
2383	ND	ONT:H28
2384	ND	O174:H28
2385	O−:H7	ONT:H7
2386	ND	O8:H14
2387	ND	ONT:H2
2388	ND	ONT:H19
2389	ND	ONT:H7
2390	ND	ONT:HNT
2391	O113:H36	O113:H21
2392	O113:H36	O113:H21
2393	ND	ONT:H6
2394	ND	O101:H6

[a] ECRC, *E. coli* Reference Center; ND, not determined.
[b] NT, nontypeable.

DISCUSSION

E. coli strains can theoretically carry any combination of the known 181 O and 53 H antigens. Therefore, serotyping is highly complex and often limited in effectiveness, resulting in strains with partial or no serotype data. As an alternative, we explored the potential of using the FDA-ECID microarray to molecularly serotype *E. coli* strains. Our study revealed several discrepancies between genotypic and serotypic assays. Traditional serotyping depends on the expression of specific phenotypes, but gene expression may be affected by physiological and environmental factors as well as by genetic mutations, resulting in the absence of gene expression. For example, it is common to isolate O157:H7 strains that are nonmotile and do not express the H7 antigen (13). Similarly, O-rough strains of O157:H7 that do not express the O157 antigen due to genetic insertions in the O157 operon have been isolated from foods (14) and clinical patients (15). In all of these instances, the strains could not be characterized by serology but were effectively identified and molecularly serotyped by genotypic assays (16, 17). Thus, the broad application of a fully discrete molecular serotype signature may inherently redefine the practice of serotyping and identifying bacteria based on phenotypic expressions.

Analysis of the DEC reference panel showed that the array correctly identified the O and H types in 97% and 98% of the strains, respectively. There were two strains that gave a discrepant O type by the array compared to serology, but the target gene sequences of these strains concurred with the array data; furthermore, considering that the array panel included both the O-antigen flippase gene (*wzx*) and the O-antigen polymerase gene (*wzy*) for each O type and that both were negative for the supposed O type determined by serology, it is likely that these strains were mislabeled or were mistyped by serology. Analysis of the H types in the DEC panel showed that an H21 strain was typed as H8 by the array. However, this strain, DEC 14a, was shown by the array and sequence analysis to be of the O86 serogroup and not the expected O128 serogroup, a further indication that this strain was possibly mislabeled. As discussed below, there were six STEC strains that were erroneously H typed by serology but were correctly identified by the array. So, it is possible that the H type of this DEC strain was also mistyped by serology.

Since the FDA-ECID array was shown to be effective for serotyping, it was used to examine a panel of produce STEC strains,

many of which were nontypeable or for which there were only partial serotypes. Serology and the array identified O types in 37% and 50% of the STEC strains, respectively. But the two assays agreed on only 60% of the O-type identifications, so there were many discrepancies. There were five strains that had an O type by serology but could not be identified by the array. Two of these were strains of the O76 and O88 serogroups, and since neither of these O types is represented on the array, they could not be detected. The others were two strains of O1 and one strain of O107, O types that are both redundantly represented on the array. None of the O1 or O107 *wzx* or *wzy* probes reacted with these strains, so perhaps the O types of these three strains may have been mistyped by serology.

There were four STEC strains (2337, 2365, 2377, and 2379) that were serologically determined to have an OX serotype (Table 2). The OX designation was introduced years ago to denote unclassified *E. coli* strains that would not react with standard O antisera (18). In an attempt to determine some serological identity, a panel of 45 OX antisera was developed. Any unclassified strain that reacted with one of these sera was designated with that OX number, but its true O type is unknown. Array analysis of the four OX strains provided no further information on the O type for three strains, but strain 2379 was found to have the antigen sequences of *Shigella boydii* type 9 (B9). The array found six additional strains with B9 antigen sequences, results that were confirmed using allele-specific PCR of the *wzx* and *wzy* genes (data not shown). These findings are not surprising since *E. coli* and *Shigella* are very closely related genetically and share many O antigens (19), but it was interesting that B9 strains comprised 10% (7/73) of the produce STEC strains examined.

There were several strains that could not be O serotyped, but the array was able to identify or provide partial serological identity of these strains. Three of these untyped strains belong to the O17/O44/O73/O77/O106 group of O types. Strains within this group share identical backbone O-subunit structures but exhibit multiple O-antigen types due to glucosylation of the common backbone by proteins, which presumably are encoded on resident prophages (20). There were 23 STEC strains that could not be O typed by either serology or the array. Among these were four O-nontypeable:H52 strains (2322, 2323, 2328, and 2329) that were previously characterized and found to carry virulence genes of both STEC and ETEC and belong to a unique clonal group (21). We had hoped that the array could genotype the O antigens of these unique strains that were untypeable by serology, but unfortunately the O types of these strains remain unidentified.

The array contains the entire panel of H-type gene targets, and, as expected, it performed much better and identified the H types of 97% of the STEC strains compared to 65% by serology. Two of the strains that were mistyped were identified by serology to be O113:H36 strains, but the array and sequence analysis confirmed these to be O113:H21 strains. This serological mistyping is of public health significance as STEC O113:H36 strains have not been reported to cause human disease, but O113:H21 strains have been implicated in outbreaks of severe illness worldwide (22). The mistyping also has an impact on food safety as it showed that there were actually five O113:H21 strains being isolated from spinach instead of three, as previously reported (5).

There were three strains (2325, 2331, and 2390) that had no O type by serology or the array but were found to have *fliC* sequences of *E. cloacae*. Based on biochemical profiles, all three strains were determined to be *Enterobacter* sp. isolates, which would account for the absence of an *E. coli* O type. A previous study (2) had shown that two of these strains did not carry Shiga toxin (*stx*) genes, so both were not only misidentified to be STEC but were also mistaken to be *E. coli*. The third strain (2325) was found to possess the genes for the Stx2a subtype (2). While unusual, *Enterobacter* species that produce Stx have been isolated and reported by other investigators (23, 24). The genes that encode Stx reside on bacteriophages, which can be induced and transferred between strains or even species.

In conclusion, the FDA-ECID array was effective in molecularly serotyping produce STEC strains, many of which could not be serotyped or had only partial serotypes. The array contains 80% of the known O-type targets but was able to identify strains that were serologically untypeable and to outperform serology that used the entire panel of O-type antisera. Moreover, it correctly identified the H types of almost all the produce STEC isolates (96%), including six strains that were mistyped by serology. The FDA-ECID array assay is rapid, simple, and economical (~$100/isolate). Once DNA is extracted from an isolate, a result/serotype is obtained within 24 h and requires less than 2 h of actual hands-on time, as opposed to traditional serology, which typically takes a few weeks to perform. Furthermore, the FDA-ECID assay simultaneously provides information on tens of thousands of other *E. coli* genetic markers (SNPs, genes, and virulence factors) that are also represented on this microarray platform. The design details and the results obtained from these additional biomarkers are the focus of a different manuscript that is currently in preparation and, therefore, not presented here. These advantages make the FDA-ECID array an attractive alternative to serotyping *E. coli* and also a viable tool for determining the serological identity of strains that are untypeable by serology.

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