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Use of *fimH* Single-Nucleotide Polymorphisms for Strain Typing of Clinical Isolates of *Escherichia coli* for Epidemiologic Investigation▽

Rubens C. S. Dias,[1,2] Beatriz M. Moreira,[3]* and Lee W. Riley[2]

Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-902, Brazil[1]; School of Public Health, University of California, Berkeley, California 94720-7360[2]; and Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-902, Brazil[3]

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Strain typing methods that compare electrophoresis banding patterns are commonly used but are difficult to standardize and poorly portable. Multilocus sequence typing (MLST) is a sequence-based alternative, but it is not practical for large-scale epidemiological studies. In the present study, the usefulness of *fimH* single-nucleotide polymorphisms (SNPs) for *Escherichia coli* typing was explored. *fimH* SNPs were determined for 345 *E. coli* clinical isolates (including 3 reference strains) and compared to PCR-based ECOR (*E. coli* reference collection) phylogrouping. The *fimH* gene could be amplified for 316 (92%) of the 345 isolates. *fimH* SNP analysis found 46 distinct terminal groups in the nucleotide sequence-based phylogenetic tree (*fimH* types). A subset of the *E. coli* isolates (162 clinical isolates and the 3 reference strains) were compared by *fimH* type, PCR phylogroup, and MLST. These isolates fell into 27 *fimH* types and 18 MLST clonal complexes (CCs) that contained 2 to 28 isolates per complex. The combination of PCR phylogroup and *fimH* type corresponded to a single CC for 113 (68%) isolates and 2 or 3 CCs for the other 52 (32%) isolates. We propose that the combination of PCR phylogrouping and *fimH* SNP analysis may be a useful method to type a large collection of clinical *E. coli* isolates for epidemiologic studies.

Molecular strain typing methods have enhanced our understanding of the epidemiology of many infectious diseases by contributing to the characterization of new modes of transmission, vehicles, and risk factors for infections. Recently, a new understanding about the epidemiology of community-acquired urinary tract infections (UTI) emerged from the systematic strain typing analysis of uropathogenic *Escherichia coli* (UPEC). Community-acquired UTI typically occurs as an endemic infection. In 2001, Manges and colleagues identified clusters of UTI on 3 different college campuses in the United States caused by an *E. coli* clonal group that was designated CgA based on a characteristic enterobacterial repetitive intergenic consensus (ERIC)-PCR electrophoresis banding pattern (14). Additional strain typing by *E. coli* reference collection (ECOR) phylogenetic grouping, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), serotyping, and virulence profiling revealed that the CgA strains belonged to an identical or a very closely related lineage of *E. coli* (9, 14, 20). In 2009, in Rio de Janeiro, Brazil, CgA was also found to comprise the largest clonal group observed among women with community-acquired UTI (4).

Although reference laboratory surveillance systems using PFGE, such as PulseNet, have been highly successful, the analysis of multiple electrophoresis banding patterns is subject to interlaboratory variability and is not amenable to simple standardization for portability and the creation of public databases. MLST was developed as an attractive sequence-based genotyping technique because it provides reproducibility, comparability, and transferability between laboratories. Although MLST seems to be an excellent technique, it is still impractical for large-scale epidemiological studies. In a previous study, Tartof et al. explored *fimH* single-nucleotide polymorphism (SNP) analysis as a screening test for the epidemiological study of UPEC (21). FimH is a specific adhesin located at the tip of type 1 fimbriae that determines mannose-sensitive binding of bacteria to target cells (11). *fimH* SNPs were compared with MLST for 34 *E. coli* isolates from urine, blood, animals, and water, belonging to 14 distinct sequence types (STs), including 22 CgA isolates and strains K-12 and CFT073 (21). The two techniques (MLST *versus fimH* SNP analysis) showed similar discriminatory powers; however, the study used a collection of well-defined, selected *E. coli* isolates. The performance of *fimH* SNP analysis on population-based clinical isolates of *E. coli* is not known. In the present study, we explored *fimH* SNP analysis as a typing tool for a large collection of community- and hospital-associated extraintestinal *E. coli* isolates and compared this typing method to ECOR phylogrouping and MLST.

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MATERIALS AND METHODS

Bacterial isolates. Between 2001 and 2006, 342 *E. coli* isolates from clinical samples were collected in Rio de Janeiro, Brazil. One hundred twenty-seven and 12 isolates were recovered from urine samples of women and men, respectively, with UTI in the community of Rio de Janeiro (4, 5); 42 hospital isolates were recovered from different clinical specimens (including blood, lower respiratory tract, surgical site, normally sterile secretions and tissues, catheter tip, and other sources) of patients (22 men and 20 women) at a public university-affiliated hospital (3); and 120 and 41 isolates were recovered from urine samples of hospitalized female and male patients, respectively (unpublished data). Only one

* Corresponding author. Mailing address: Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Bloco I. Av. Carlos Chagas Filho, 373, Cidade Universitária, Rio de Janeiro 21941-902, Brazil. Phone and fax: 55-21-25608344, ext. 131. E-mail: bmeurer@micro.ufrj.br.

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isolate per patient was included. *E. coli* reference strains ATCC BAA-457 (CgA), CFT073, and K-12 were also studied for comparison.

DNA isolation. *E. coli* isolates were obtained from strains stored in suspensions containing skim milk (10%) and glycerol (10%) at −80°C. Isolates were incubated at 37°C on nutrient agar plates overnight. Single colonies were picked and inoculated into 2 ml of nutrient broth and further incubated in a shaking incubator for 16 to 18 h at 37°C. A 500-μl suspension of bacteria was centrifuged, and chromosomal DNA was extracted from the bacterial pellet suspended in 100 ml of sterile ultrapure water by boiling for 10 min. After thermal lysis, the supernatant was transferred to a new microtube and kept at −20°C.

***fimH* single-nucleotide polymorphisms.** *fimH* SNP analysis was performed as reported previously (21), with modifications. The primers used for PCR amplification and partial *fimH* gene sequencing were FimH-f (5′-CGAGTTATTACC CTGTTTGCTG-3′) and FimH-r (5′-ACGCCAATAATCGATTGCAC-3′). Both strands of the 878-bp PCR-amplified fragment (located at bp 7 to 884 of *E. coli* sequence NC000913 [GenBank]) were sequenced. After visual inspection and editing with BioEdit (version 7.0.9.0), fragments of 424-bp *fimH* sequences (located at bp 401 to 824 of *E. coli* sequence NC000913 [GenBank]) were compared to the sequence of *E. coli* K-12.

Multilocus sequence typing. We performed MLST using a standardized protocol for *E. coli* maintained at the MLST databases at the ERI website (http://mlst.ucc.ie/) of University College Cork, based on the seven housekeeping genes *adk*, *fumC*, *icd*, *purA*, *gyrB*, *recA*, and *mdh*. The primers used for amplification and sequencing of both DNA strands were same as those described on the MLST website, except for the *adk* forward primer. A 704-bp *adk* fragment was obtained, with primer adkF-f2 (5′-CTCGCCATTAACCGTTTCA-3′), instead of the 583-bp fragment described in the MLST website. Briefly, amplifications were carried out in a total volume of 50 μl. Each reaction mixture contained 0.2 mM each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 0.2 μM each primer, 2.5 U Ampli*Taq* Gold (Applied Biosystems), and 2 μl template DNA. The reaction conditions were 2 min of initial denaturation at 95°C followed by 30 1-min cycles of denaturation at 95°C; 1 min of annealing at 54°C for *adk*, *fumC*, *icd*, and *purA* or 60°C for *gyrB*, *mdh*, and *recA*; 2 min of extension at 72°C; and a final extension step of 5 min at 72°C.

PCR-based phylotyping. Phylogenetic grouping of *E. coli* isolates was assessed by a previously reported triplex-PCR-based assay (2, 6). The results allowed the classification of isolates into one of the four major phylogroups (A, B1, B2, or D). *E. coli* strains ATCC BAA-457 (CgA), CFT073, and K-12 were used as controls for groups D, B2, and A, respectively.

Sequencing and sequence analysis. Amplicons were purified with a QIAquick gel extraction kit (Qiagen, Inc., Valencia, CA), and DNA sequencing of both strands was carried out at the DNA Sequencing Facility of the University of California, Berkeley, CA. The forward and reverse PCR primers were used for sequencing of the *fimH* and housekeeping genes. The facility runs a 25-cycle sequencing reaction with the following program: 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. Forward- and reverse-strand DNA sequence traces were visually inspected and edited with BioEdit Sequence Alignment Editor, version 7.0.9.0 (7).

The finished sequences were aligned by using ClustalW Multiple Alignment in BioEdit. The alignment data were assessed by bootstrap analyses based on 1,000 resamplings. The 424-bp *fimH* gene fragment alignment of the 342 clinical *E. coli* isolates and *E. coli* reference strains was imported into MEGA4 (19) for phylogenetic analysis and construction of trees. For the same strains, an MLST alignment was made from the concatenated sequences of the seven housekeeping genes (3,423 bp). To conserve computing time, duplicate concatenated sequences were removed from the input sample. Aligned sequences were examined for molecular evolutionary relationships by the neighbor-joining method (17). The significance of the branching order was evaluated by bootstrap analysis with 500 replicates. The evolutionary distances were computed by the Kimura 2-parameter method (10).

GenBank accession numbers. The *fimH* gene sequences analyzed (partial coding sequence [CDS] located at bp 44 to 862 of *E. coli* sequence NC_000913 [GenBank]) were deposited in GenBank under accession nos. GQ486876 to GQ486913 and GQ487043 to GQ487188 (hospital isolates), GQ486914 to GQ487042 (community isolates), and GQ487189 to GQ487191 (reference strains ATCC BAA-457 [CgA], K-12, and CFT073, respectively).

RESULTS

We first examined SNPs of the 424-bp *fimH* sequence of all *E. coli* isolates to estimate more precisely the potential use of this gene as a typing tool. Twenty-nine (8%) of the 345 *E. coli* isolates included in the study were *fimH* negative (10 [7%] community isolates and 9 [4%] hospital isolates).

Each DNA sequence was compared to that of *E. coli* K-12. Among the 345 isolates, 46 distinct *fimH* allelic variants were observed (Fig. 1), with 53 unique mutations (SNPs) at 49 polymorphic sites. All mutations were point substitutions; 14 (26%) were transversions, 39 (74%) were transitions, 16 resulted in amino acid replacements, and 37 were silent substitutions. Among the amino acid replacements, only four were caused by transversion. Seventeen of the 53 SNPs were singletons (observed in only one *fimH* type), with 8 amino acid replacements.

We analyzed the distribution of *fimH* variants with an unrooted phylogram (Fig. 1). In this tree, 46 distinct terminal groups representing distinct allelic variants were named f-1 (*E. coli* K-12 type) to f-46. GenBank accession numbers for each *fimH* type are shown in Table 1. No specific node was observed on the tree according to the source or date of isolation. However, some *fimH* types were found only among hospital (f-16, f-18, f-22 to f-28, and f-36 to f-46) or community (f-17 and f-29 to f-35) isolates. The *fimH*-negative phenotype was named f-0.

The correlation between *fimH* type and MLST was further explored in a subset of 165 isolates comprising the more diverse collection of UPEC isolates obtained in the community (139 isolates, including 10 *fimH*-negative strains), the three reference strains, and all isolates (29 of the isolates already included in the collection of community isolates and 23 additional hospital isolates) included in the largest group sharing identical *fimH* sequences. This way, we could select the isolates with the highest possible diversity and the largest possible cluster.

The 165 isolates were classified into 51 distinct STs, including 16 single-locus (13) or double-locus (3) variants (SLV and DLV, respectively) of known STs. Five new STs were observed (ST697, ST706, ST827, ST828, and ST1393). Of these five STs, three included one new allele each (*mdh101*, *icd154*, or *icd215*). These data were deposited into the *E. coli* MLST website database (http://web.mpiib-berlin.mpg.de). The isolates were then classified into clonal complexes (CCs), defined as groups of isolates with an identical ST or an SLV or DLV from another ST within the CC (12). A total of 149 (90%) of the 165 isolates grouped into 18 CCs with 28 to 2 isolates per CC, numbered from "I" (CC with 28 isolates) to "XVIII" (CC with two isolates) (Fig. 2). The remaining 16 (10%) isolates belonged to a distinct ST each. The 10 *fimH*-negative strains fell into 3 MLST CCs and 3 STs not included in a CC.

The 165 isolates were discriminated into 27 *fimH* types. The *fimH* type and MLST assignments were then compared. Each of 19 *fimH* types corresponded to a single CC or an ST not included in a CC. The other 8 *fimH* types corresponded to multiple CCs or STs; these CCs and STs also included several different *fimH* types.

The PCR phylogrouping discriminated the 165 isolates into 75 B2, 59 D, 20 A, and 11 B1 ECOR groups. Of 27 *fimH* types, 20 were observed to belong to a single PCR phylogroup (f-20 and f-32 to phylogroup A; f-10, f-11, f-15, f-21, and f-35 to B1; f-5, f-13, f-19, f-33, and f-34 to D; and f-2, f-3, f-8, f-14, f-17, f-29, f-30, and f-31 to B2); the other 7 *fimH* types belonged to 2 (f-7, f-9, and f-12), 3 (f-6), or all 4 PCR phylogroups (f-0, f-1, and f-4) (Table 2). All isolates within each ST belonged to a single PCR phylogroup (Fig. 2). In addition, *fimH* type-MLST combinations within each phylogroup were also unique (Table

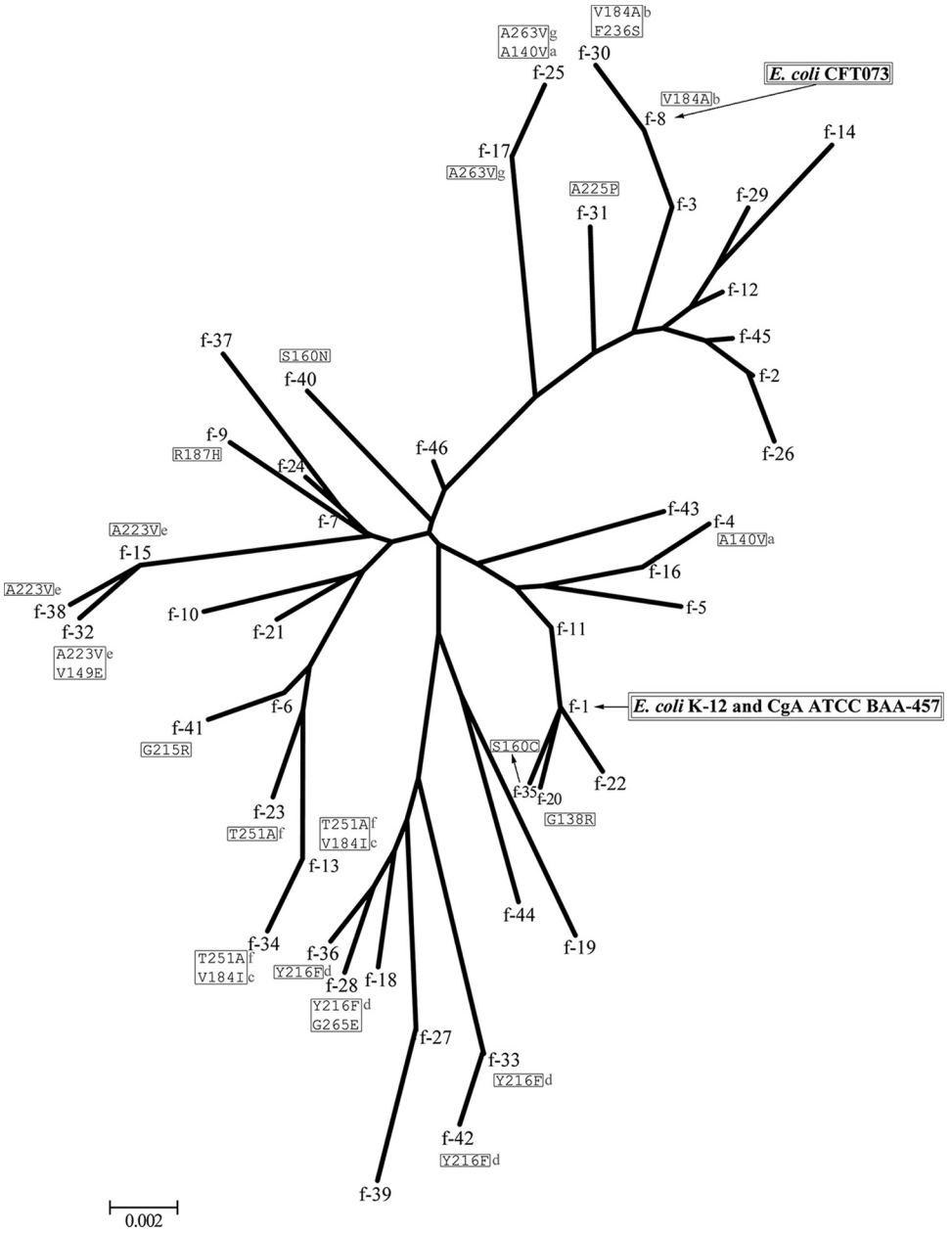

FIG. 1. Phylogenetic tree derived from 46 partial *fimH* sequence variants found in 342 clinical *E. coli* isolates and *E. coli* reference strains K-12 (*fimH* type f-1), CFT073, and ATCC BAA-457 (CgA), determined by the neighbor-joining distance method using the Kimura 2-parameter model. *fimH* sequences were analyzed by comparison with the sequence of *E. coli* K-12. Boxes contain amino acid changes that resulted in 17 FimH protein variants deduced from the analysis of the nucleotide sequences. Replacements of the same amino acid in the same position independently acquired are distinguished by lowercase letters to the left of boxes.

2). On the other hand, the *fimH* type-PCR phylogroup combinations corresponded to a single CC (or an ST not included in a CC) for 113 (68%) isolates and to 2 or 3 CCs (or STs not included into a CC) for the other 52 (32%) isolates.

A new phylogenetic tree was built with the 155 concatenated MLST sequences (corresponding to 46 STs) of the *fimH*-positive isolates. This new tree was then rebuilt with the 26 *fimH* type sequences added to the concatenated MLST sequences. As expected, the tree containing *fimH* was more discriminatory (with 57 terminal groups) than the tree without *fimH* (with 46 terminal groups). The nodes corresponding to the PCR phylogroups in the tree containing *fimH* overlapped the respective nodes in the tree without *fimH*. The internal nodes showed a few changes, but, with only one exception (isolates included in CCII), the arrangements within CCs were maintained (data not shown).

DISCUSSION

In a previous study, *fimH* SNP analysis was explored as a possible screening tool to type UPEC (21). For a select and

TABLE 1. Single-nucleotide polymorphism(s) and gene accession numbers for each *fimH* type

fimH type	*fimH* SNP(s)[a]	GenBank accession no.
f-1	None (*E. coli* K-12 reference sequence)	GQ487190
f-2	C411G, G414A, T534C, C546T, C577T, A603G, T714C, A717G, G807A	GQ486937
f-3	C411G, G414A, T534C, C546T, C577T, C654A, T714C, A717G, G807A	GQ486930
f-4	C419T (A140V), T534C, A603G, G807A	GQ486914
f-5	G420A, C546T, A603G, G807A	GQ486919
f-6	T534C, C546T, T714A, A717G, G795A, G807A	GQ486928
f-7	T714A, A717G, G807A	GQ486915
f-8	C411G, G414A, T534C, C546T, T551C (V184A), A603G, C654A, T714C, A717G, G807A	GQ487191
f-9	C411T, G560A (R187H), T714A, A717G, G807A	GQ486920
f-10	C546T, G645A, G702C, T714A, A717G, G807A	GQ487018
f-11	G807A	GQ487032
f-12	C411G, G414A, T429A, T534C, A603G, T714C, A717G, G807A	GQ486933
f-13	T534C, C546T, G550A (V184I), T714A, A717G, A751G (T251A), G795A, G807A	GQ486923
f-14	C411G, G414A, T429A, T534C, C577T, A603G, C640T, G807A	GQ486916
f-15	C489T, C639T, C668T (A223V), T714A, A717G, G807A	GQ486948
f-16	T534C, A603G, G807A	GQ487043
f-17	C411G, G414A, T450A, C546T, A603G, T714A, A717G, C788T (A263V), G807A	GQ486952
f-18	C588T, G702C, T714A, A717G, G741A, G807A	GQ487120
f-19	C432T, C471T, C489T, T534C, A603G, G702C, T714C, A717G, G807A	GQ486932
f-20	G412A (G138R)	GQ486964
f-21	G420A, C546T, T714A, A717G, G807A	GQ486982
f-22	T591A	GQ487114
f-23	C489T, C546T, T714A, A717G, A751G (T251A), G795A, G807A	GQ487129
f-24	C699T, T714A, A717G, G807A	GQ487091
f-25	C411G, G414A, C419T (A140V), T450A, C546T, T714A, A717G, C788T (A263V), G807A	GQ487063
f-26	C411G, G414A, T429A, T534C, C546T, C577T, T714C, A717G, G807A	GQ487061
f-27	T450A, C546T, C588T, G702C, T714A, G807A	GQ487078
f-28	C588T, A647T (Y216F), T714A, A717G, G794A (G265E), G807A	GQ487085
f-29	C411G, G414A, T429A, T534C, A603G, C640T, T714C, A717G, G807A	GQ486924
f-30	C411G, G414A, T534C, C546T, T551C (V184A), A603G, C654A, T707C (F236S), T714C, A717G, G807A	GQ486970
f-31	C411G, G414A, T534C, A603G, G673C (A225P), A717G, G807A	GQ486999
f-32	T446A (V149E), C489T, C639T, C668T (A223V), T714A, A717G, G807A	GQ487007
f-33	C432T, C489T, T534C, C588T, T591C, A597G, A603G, A647T (Y216F), T714A, A717G, G807A	GQ486959
f-34	T534C, C546T, G550A (V184I), T618A, T714A, A717G, A751G (T251A), G795A, G807A	GQ487014
f-35	A478T (S160C)	GQ486938
f-36	C588T, A647T (Y216F), G702C, T714A, A717G, G807A	GQ487083
f-37	T558C, C699A, T714A, A717G, G795A, G807A	GQ487051
f-38	C489T, C639T, C668T (A223V), C693A, T714A, A717G, G807A	GQ487093
f-39	G408T, T450A, C546T, C588T, G702C, T714A, A792G, G807A	GQ487109
f-40	G479A (S160N), C657T, T714A, A717G, G807A	GQ487156
f-41	T534C, C546T, G643A (G215R), T714A, A717G, G795A, G807A	GQ487138
f-42	G408T, C432T, C489T, T534C, C588T, A647T (Y216F), T714A, A717G, G807A	GQ487157
f-43	G702C, T714A, A792G, G807A	GQ487155
f-44	G408T, C432T, C489T, T534C, T714A, A717G, G738A, G807A	GQ487117
f-45	C411G, G414A, T534C, C577T, T714C, A717G, G807A	GQ486894
f-46	C546T, T714A, A717G, G807A	GQ486889

[a] *fimH* sequences were analyzed by comparison with the sequence of *E. coli* K-12. Only SNPs located at bp 401 to 824 of *E. coli* sequence NC_000913 (GenBank) are shown. Amino acid replacements are shown in parentheses. The underlined *fimH* SNPs are nucleotide replacements found in only one study isolate (singleton).

small collection of *E. coli* isolates, the *fimH* SNP analysis showed a discriminatory power similar to that of MLST (21). In the present study, we explored the usefulness of this typing method for a larger and population-based collection of *E. coli* isolates. Here we found that *fimH* SNP analysis showed a lower discriminatory power than MLST. Furthermore, the *fimH* type assignments were inconsistent with those made by MLST. This finding indicates that *fimH* SNP analysis is not useful as a screening tool in a population-based sample of clinical isolates of *E. coli*. Nevertheless, when the *fimH* type was combined with the PCR phylogroup, the combination formed unambiguous types which correlated well with the MLST CCs. Specific *fimH* type-PCR phylogroup combinations corresponded to 1 to 3 CCs (or single STs not included in CCs) for the entire collection of isolates studied. Thus, although the combination was still less discriminatory than MLST, the application of this combination test could substantially reduce the number of isolates that need to be tested by MLST. On the other hand, MLST itself may be less discriminatory than PFGE. In the study of UPEC, isolates included in one ST are usually also clustered by PFGE with at least 68% similarity (4, 15, 20).

In conclusion, *fimH* SNP analysis together with PCR phylogrouping seems to be a simple strain typing tool for epidemiological studies of *E. coli* isolates. The sequence analysis in-

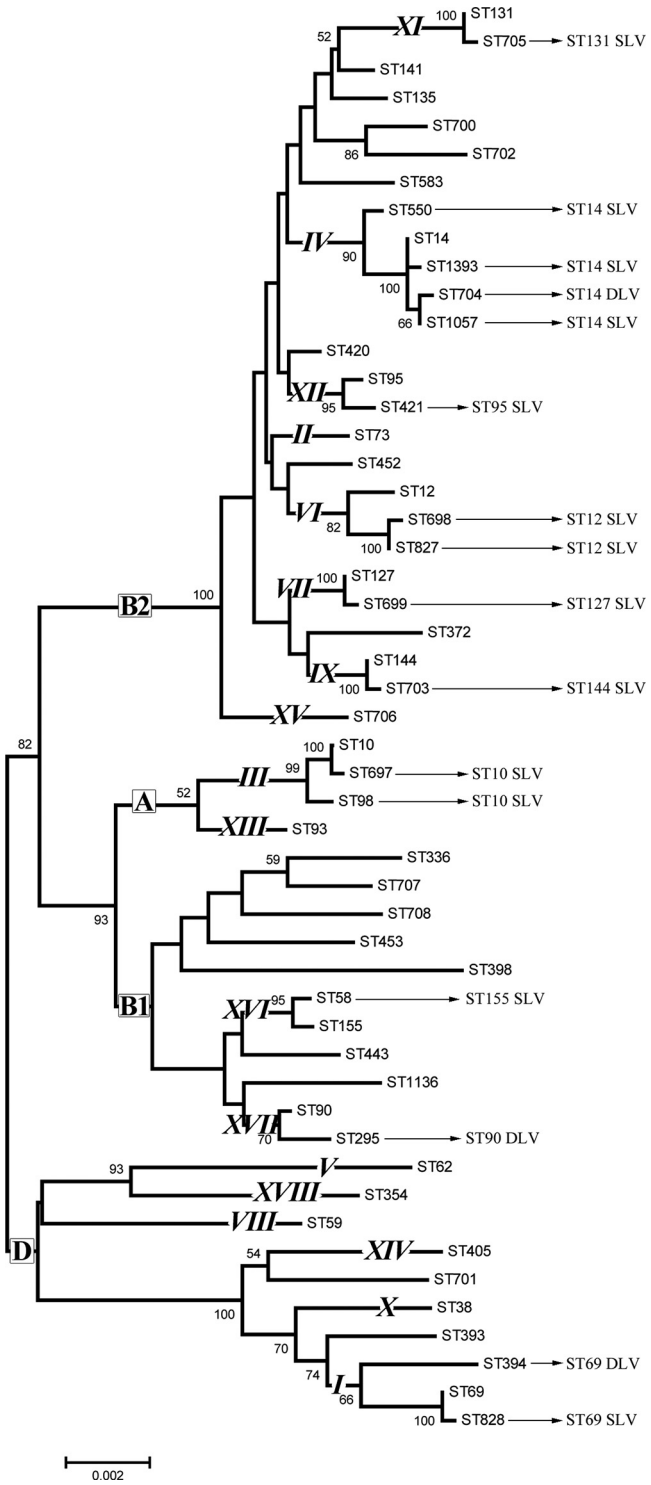

FIG. 2. Phylogenetic tree of 166 *E. coli* isolates (including reference strains K-12, CFT073, and ATCC BAA-457 [CgA]) derived from concatenated sequences of the seven standard MLST genes (described in the text), determined by the neighbor-joining distance method using the Kimura 2-parameter model. Duplicate concatenated sequences were removed from the input sample. B2, A, B1, and D are PCR phylogenetic groups. Numbers within the tree indicate the occurrence (%) of the branching order in 500 bootstrapped trees. Only values above 50 are shown. Roman numerals indicate clonal complexes.

TABLE 2. Distribution of *fimH* types in the phylogenetic groups, multilocus sequence types, and clonal complexes

Phylogroup and *fimH* type[a]	MLST(s)	CC
A		
f-1*	ST98/ST10 SLV	III
	ST10	III
f-4	ST10	III
f-6	ST10	III
	ST697/ST10 SLV	III
f-7	ST10	III
f-20	ST10	III
f-32	ST10	III
f-0	ST93	XIII
B1		
f-1	ST295/ST90 DLV	XVII
f-4	ST336	—[b]
f-7	ST453	—
	ST708	—
	ST1136	—
f-10	ST155	XVI
f-11	ST58/ST155 SLV	XVI
f-15	ST443	
f-21	ST707	
f-35	ST90	XVII
f-0	ST398	—
D		
f-1**	ST69	I
	ST828/ST69 SLV	I
f-4	ST393	—
f-5	ST62	V
f-6	ST59	VIII
f-9	ST38	X
f-12	ST38	X
f-13	ST405	XIV
f-19	ST354	XVIII
f-33	ST701	—
f-34	ST405	XIV
f-0	ST38	X
	ST394/ST69 DLV	I
B2		
f-1	ST14	IV
	ST704/ST14 DLV	IV
	ST1057/ST14 SLV	IV
	ST1393/ST14 SLV	IV
	ST827	VI
	ST698/ST827 SLV	VI
	ST12/ST827 SLV	VI
	ST95	XII
f-2	ST127	VII
	ST699/ST127 SLV	VII
f-3	ST73	II
	ST372	—
	ST702	—
f-4	ST144	IX
	ST703/ST144 SLV	IX
	ST421/ST95 SLV	XII
f-6	ST12/ST827 SLV	VI
	ST700	—
f-8***	ST73	II
f-9	ST73	II
	ST550/ST14 SLV	IV
f-12	ST141	—
f-14	ST706	XV
f-17	ST131	XI
	ST705/ST131 SLV	XI
f-29	ST420	—
f-30	ST73	II
f-31	ST583	—
f-0	ST135	—
	ST452	—

[a] Phylogroup was determined by PCR (2, 6). *fimH* type was determined for study isolates and *E. coli* reference strains K-12 (*), ATCC BAA-457 (**), and CFT073 (***). f-0, *fimH* PCR negative.

[b] —, ST not included in a CC.

volves a single small (424 bp) DNA fragment. The phylogrouping analysis involves comparison of only up to three bands of known molecular weight; it does not involve analysis of electrophoresis banding patterns, as does PFGE or PCR finger-

printing. This analysis requires only 2 sets of primers as opposed to 7 with MLST. Although this typing tool may not replace MLST, this method is suitable for screening large collections of *E. coli* isolates, allowing for the rapid identification of STs or CCs. Furthermore, concatenated MLST and *fimH* sequences were found to be more discriminatory than concatenated sequences based on MLST alone. However, the epidemiological usefulness of the clusters formed by *fimH* typing needs further studies that include detailed patient clinical and epidemiologic information.

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