INFECTION AND IMMUNITY, June 1997, p. 2011–2018 Vol. 65, No. 6
0019-9567/97/$04.00+0

Distribution of *drb* Genes Coding for Dr Binding Adhesins among Uropathogenic and Fecal *Escherichia coli* Isolates and Identification of New Subtypes

LIXIN ZHANG,[1] BETSY FOXMAN,[1] PAT TALLMAN,[1] EDUARDO CLADERA,[1] CHANTAL LE BOUGUENEC,[2] AND CARL F. MARRS[1]*

Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan 48109,[1] *and Unité de Pathogénie Bactérienne des Muqueuses, Institut Pasteur, Paris, France*[2]

Received 21 November 1996/Returned for modification 15 January 1997/Accepted 13 March 1997

The Dr family of related adherence structures, some fimbriated and others afimbriated, bind to decay-accelerating factor molecules on human cells. Dr is associated with recurring urinary tract infection (UTI), but the distribution of Dr subtypes among uropathogenic *Escherichia coli* causing UTI among otherwise healthy women has yet to be described. A total of 787 UTI and fecal *E. coli* isolates from college women were screened for the presence of Dr sequences (*drb*). Fifteen percent of UTI strains were *drb* positive, compared to 5% of fecal strains. The adhesin (E gene) subtype of each *drb*-positive strain was determined by type-specific PCR followed by restriction enzyme analysis. Among 78 *drb*-positive strains, we found 14 (18%) *afaE1*, 1 (1.3%) *afaE2*, 1 (1.3%) *afaE3*, 9 (12%) *draE*, 9 (12%) *draE-afaE3* hybrid, 1 (1.3%) *daaE*, 32 (41%) *afaE5*, 4 (5.1%) F131 E gene-like, and 7 untypeable strains. All untypeable E genes were cloned and sequenced, revealing four additional new classes of E genes, including two similar to the previously identified nonfimbrial E series. While a great range of diversity exists among the E genes, restriction fragment length polymorphism analysis demonstrated that all of these *drb* operons share a highly conserved gene structure. The most common subtype, *afaE5*, occurred three times as often among UTI than fecal strains. Over half of the *drb*-positive strains and 80% of those positive for *afaE5* have the same virulence signature (positive for *aer*, *kpsMT*, *ompT*, and *fim*), suggesting an association of this profile with UTI pathogenesis.

Bacterial adherence to host cells is a crucial step in the initiation of various infectious diseases (9, 24). An array of adhesins including type 1, P, S, and G fimbriae as well as adhesins of the Dr family and nonfimbrial adhesins (NFAs) have been identified in *Escherichia coli* strains causing urinary tract infection (UTI) (17, 19, 22). Adherence factors facilitate the colonization of the urinary tract and promote *E. coli* colonization and persistence in the colon or vagina, which may serve as a reservoir for ascending infection in the urinary tract (10, 22). The variety of adhesins, as well as the antigenic diversity of certain adhesins, may extend bacterial tissue tropism to different sites, facilitating the multiple stages of the pathogenic process.

Several pieces of evidence suggest that Dr adhesins play an important role in UTI pathogenesis. Dr adhesins occur more frequently among *E. coli* UTI isolates than fecal isolates (10, 22), and the presence of Dr at the time of a first UTI has been associated with an increased risk of a second UTI (13). Dr receptors, the Dr blood group antigen component of decay-accelerating factor (31, 32), are widely distributed along the urinary tract, underlining the potential importance of Dr adhesin-producing *E. coli* in ascending colonization of the urinary tract. Furthermore, a pathogenicity study using a mouse model demonstrated that a Dr hemagglutinin-positive *E. coli* strain was much more virulent in terms of causing persistent and severe UTI than its isogenic mutant (10, 18).

Structural and assembly genes required to produce adhesins of the Dr family are present in operons, each consisting of at least five genes often named A to E (a representative *afa-1* operon is shown in Fig. 1) (26). This fimbrial gene organization is quite different from that for other operons such as type 1 and P-pilus operons but are similar to that reported for the adhesin gene cluster of enteroaggregative *E. coli* (34). Other potential regulatory genes located upstream of gene A have been identified in some adhesin-encoding gene clusters (5, 16). In all but the *dra* operon (coding for Dr hemagglutinin), genes A to D encoding mostly accessory functions among the family are very similar in terms of amino acid sequence homology and functional homology. The last gene in each operon, E, encodes a protein that functions both as the major structural protein and as an adhesin molecule. Most of the sequence heterogeneity between family members occurs in the E gene. Similar genes exist in the *dra* operon, but the *dra* genes were named in reverse alphabetical order compared to their counterparts in other operons; thus, *draA* encodes the adhesin/structural protein (33). Recently, Carnoy and Moseley (7b) renamed the *dra* genes to fit the order found in the *afa* genes. In this report, we are also following this latter naming system shared by the majority of *drb* operons, in which E is the last gene in each operon.

Dr hemagglutinin, F1845, AfaE-I, and AfaE-III, have been studied in detail. Despite the similarities among them, interesting differences exist. Dr hemagglutinin, F1845, AfaE-I, and AfaE-III each bind to functionally distinct sites on decay-accelerating factor (32). Dr hemagglutinin and F1845 have fimbrial structures, while AfaE-I and AfaE-III are afimbrial. While Dr hemagglutinin and AfaE-III differ in only three amino acids, they differ in fimbriation, and Dr hemagglutinin recognizes type IV collagen as an additional receptor and is chloramphenicol sensitive; AfaE-III is not (38). The phenotypic differences among adhesin subtypes of the Dr family are probably due to sequence differences in their E genes (15, 28,

* Corresponding author. Mailing address: Department of Epidemiology, University of Michigan, Ann Arbor, MI 48109. Phone: (313) 647-2407. Fax: (313) 764-3192. E-mail: cfmarrs@sph.umich.edu.

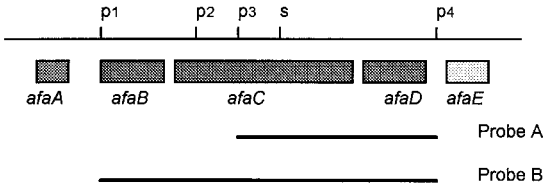

FIG. 1. Diagram of the *afa-1* gene operon (26). Letters above the lines represent positions of restriction sites: p1 to p4, *Pst*I sites; s, *Sma*I site. Boxes show positions of genes, while lines below show positions of DNA used to produce probe A and probe B.

36). The differences in chloramphenicol sensitivity for hemagglutination between AfaE-III and Dr hemagglutinin were first shown to reside in the N-terminal 54 amino acids (36) and then specifically localized to the presence of an asparagine residue and an aspartic acid residue at position 52, respectively (28). Other hybrid experiments have demonstrated that the N-terminal regions are critical for determination of fimbrial status (15). The existence of other adhesin variants such as AfaE-II and the newly discovered AfaE-V (14) and F131 (30) suggests even greater sequence heterogeneity in the genetic determinant encoding the structural adhesins of the Dr family.

Most previous studies describing the prevalence of *drb* among UTI isolates have used probes that cross-react with all members of the Dr family. Thus, the relative distribution of each subtype is unknown. Furthermore, many previous studies have had relatively small sample sizes and screened UTI isolates from laboratory collections rather than from defined populations (3, 8). In this study, we describe the frequency of the *drb* gene cluster and adhesin subtypes in a large collection of *E. coli* isolates from a well-defined population sample representative of otherwise healthy young women with first-time UTI and compare them with that of a fecal collection from healthy women of the same age group. We do similar comparisons with smaller collections of *E. coli* isolates from second UTI and from recurrent UTI (RUTI). We also describe the antigenic diversity of adhesin-encoding genes within this population and four new adhesin subtypes. The co-occurrence of *drb* genes with other virulence factors is also investigated, and virulence profiles of *drb*-positive strains are presented.

MATERIALS AND METHODS

Sample collection. We used *E. coli* isolates from three different collections. Urinary *E. coli* isolates were collected from a cohort of 304 women 18 to 40 years of age with a first UTI followed up for recurrence during the next 6 months. A total of 315 first UTI isolates and 49 second UTI isolates were collected at the University of Michigan Health Service and the University of Texas at Austin Health Service during 1992 to 1995. Twenty-eight *E. coli* isolates were collected during fall of 1994 from 27 UTI patients who had a history of RUTI, defined as at least two episodes of UTI in the prior year. Women clinically diagnosed with pyelonephritis were excluded from all UTI collections. A detailed description of recruitment methods and sociodemographic characteristics of part of the cohort are presented elsewhere (13). All urinary isolates were collected and processed as described previously (12). A total of 395 fecal *E. coli* isolates were collected from 318 consecutively sampled college women using the gynecology clinic at the University of Michigan Health Service during February and March 1996. Fecal isolates were collected using the Cultureswab Transport System (Difco Laboratories, Detroit, Mich.) and streaked for isolation on both Trypticase-soy agar with 5% sheep blood and MacConkey agar. Any phenotypically different colonies were identified and processed by using the same procedures as used for urinary *E. coli* (12).

Bacterial strains, plasmids, and culture conditions. Strain TOP10F′ (Invitrogen, San Diego, Calif.) was used as the host strain for recombinant plasmids. The following *E. coli* strains were used as controls for DNA hybridization and PCR studies: C248 (AfaE-I [*afa-1*]), C1845 (F1845 [*daa*]), and C131 (F131) were provided by S. L. Moseley of the University of Washington; and KS52 (AfaE-I [*afa-1*]), A22 (AfaE-II [*afa-2*] and AfaE-III [*afa-3*]), and AL851 (AfaE-V [*afa-5*]) were from the collection of one of us (C. Le Bouguenec). Plasmid pZErO-1 (Invitrogen) was used as the cloning vector. Plasmid pIL14 (provided by A. Labigne-Roussel, Institut Pasteur, Paris, France), containing the *afa-1* operon, was used as the source for DNA probes. All clinical and control strains were grown in Luria-Bertani (LB) broth or on LB plates at 37°C. *E. coli* HB101 carrying plasmid pIL14 was grown in LB broth or on LB plates with ampicillin (100 μg/ml). Low-salt LB agar plates with isopropylthio-β-D-galactoside (50 μg/ml) and Zeocin (50 μg/ml) were used for the selection of transformants as instructed by the manufacturer (Invitrogen).

Hemagglutination. We used the method of Nowicki et al. (32) for hemagglutinations. Briefly, normal human group O erythrocytes were washed in phosphate-buffered saline (138 mM $NaCl_2$, 1 mM NaH_2PO_4, 8 mM Na_2HPO_4) and resuspended to 3% (vol/vol) in phosphate-buffered saline with 2.5% α-methyl-D-mannoside. Hemagglutination was performed in microtiter plate wells at room temperature and at 4°C by mixing equal volumes (40 μl) of serial twofold bacterial dilutions and erythrocyte suspensions. Inhibition of mannose-resistant hemagglutination (MRHA) by chloramphenicol was tested by the addition of 10 μM chloramphenicol to an identical erythrocyte suspension prior to the assay for hemagglutination.

DNA isolation and DNA probes. Plasmid DNAs used for enzymatic manipulation were routinely prepared by an alkaline lysis procedure (21) and by using a plasmid preparation kit (Qiagen, Chatsworth, Calif.). Routine plasmid screening was done by the rapid lysis method of Kado and Liu (23). Larger amounts of purified total DNA were prepared by the methods of Hull et al. (20). DNA fragments containing *afa-1* were isolated by restriction endonuclease digestion of pIL14 plasmid DNA followed by gel electrophoresis. Either a 2.6-kb *Pst*I fragment or three *Pst*I fragments (2.6, 1.1, and 0.4 kb) were cut out, and the DNA was eluted. The purified DNA fragments were used as templates to generate digoxigenin-labeled DNA probes (Genius System kit; Boehringer Mannheim, Indianapolis, Ind.).

Dot and Southern blots. We screened for virulence factors by using dot blot hybridization as previously described (12). Both dot blots and Southern blots were hybridized to digoxigenin-labeled DNA probes, using conditions described previously (12).

Subtyping by PCR and restriction digestion. The subtype of each *drb*-positive strain was determined by PCR using a conserved primer derived from a common 5′ flanking region and a selective primer derived from the unique coding region within each known subunit gene. Table 1 lists the oligonucleotide primers used in PCR subtyping. Full-length adhesin-encoding genes of four Dr subtypes, AfaE-III, Dr hemagglutinin, AfaE-V, and F131, were amplified by a pair of primers (conserved primer and degenerate primer in Table 1). Amplification reactions were carried out with a MicroCycler (Eppendorf Inc., Fremont, Calif.). To differentiate between AfaE-III and Dr hemagglutinin, restriction patterns were compared by digestion of PCR products with enzymes *Tsp*92I and *Hsp*509I. To differentiate between AfaE-V and F131, restriction patterns were compared by digestion of PCR products with enzymes *Bam*HI and *Ava*II.

TABLE 1. Oligonucleotides used in PCR typing

Name	Oligonucleotide	Strand	Size (bp)[a]	Reference(s)
Conserved primer	5′-TTAGACCGTACTGTTGTGTTACC-3′	+		This study
AfaE-I-type primer	5′-GTAGGTGCTGTTGGTCATTTCG-3′	−	487	28
AfaE-II-type primer	5′-GTTTCCCAGTAGACTGGAATGAAGC-3′	−	375	27[b]
AfaE-III/Dr-type primer	5′-ACCATTGTCGGTCGTCCAGGC-3′	−	408	28, 35
AfaE-V/F131-type primer	5′-AGCATCGGCGCGGTATACGGT-3′	−	429	27[c], 30
F1845-type primer	5′-AGTTTGTCATGGTTGCGGCTAG-3′	−	353	6
Degenerate primer	5′-TNAANTNNNCCAGNANCCNCC-3′	−	~530	This study

[a] Distance of PCR fragment generated by using the conserved primer plus each addition primer.
[b] The nucleotide sequence accession number for *afaE2* is X85782.
[c] The nucleotide sequence accession number for *afaE5* is X91748.

Cloning and sequencing. Two approaches were used for cloning E genes of the new subtypes. A conserved primer and a degenerative primer (Table 1) were used in PCR to amplify E gene sequences from strains with untypeable subtypes. The amplified DNA was cloned into plasmid vector pZErO-1. Two independent recombinant molecules constructed from the insertion of each PCR product were selected. Alternatively, DNA from these strains was isolated and digested with *Sma*I, and the appropriate DNA fragment was identified by Southern hybridization with the DNA probe A (Fig. 1) and eluted from the gel after agarose gel electrophoresis. The DNA fragments were then purified and cloned into the plasmid vector. The recombinant plasmid DNAs were prepared by using a plasmid kit (Qiagen). The sequence of the double-stranded DNA was determined at the University of Michigan Molecular Biology Core Facility by an Applied Biosystems model 373A automated sequencer. When required, new internal oligonucleotide primers were synthesized on the basis of the determined sequence and used as new sequence primers. GenBank accession numbers for these sequences are U87540 and U87790.

Other genetic methods. Restriction enzyme digestion, nuclease treatment, and ligation were performed by standard procedures according to the methods of Maniatis et al. (29) and the manufacturers' instructions. Transformation was accomplished by calcium chloride preparation of competent cells. DNA was recovered from agarose gels by using an Elu-Quick DNA purification kit (Schleicher & Schuell, Keene, N.H.). PCR-amplified DNA was purified by using by a QIAquick Spin PCR purification kit (Qiagen). The *afaD* genes were amplified by PCR using a pair of primers (5′-CTGAGCTCCACCTGGAGAGC-3′; 5′-TTC CTGTGGCACCACACAGG-3′) derived from *afaD3* internal sequences (15).

Data analysis. Differences between groups were tested by using χ^2, a test for equality of two proportions, or, for smaller sample sizes, the Fisher exact test. Excel software (Microsoft, Redmond, Wash.) was used for data entry. Software packages from DNAStar (Madison, Wis.) were used for DNA sequence and amino acid sequence analysis and comparison. For amino acid comparisons, a Lipman-Pearson protein alignment (Ktuple = 2; gap penalty = 4; gap length penalty = 12) was initially used to align the pilin proteins. The number of perfect matches divided by the length of the longest pilin times 100 was defined as percent identical, while the number of perfect matches plus the number of conservative amino acid substitutions divided by the length of the longest pilin times 100 was defined as percent similar.

RESULTS

Detection of *drb* sequences among *E. coli* isolates. A total of 787 *E. coli* strains from otherwise healthy college women with a first UTI, a second UTI, or RUTI and from fecal specimens of college women without UTI were screened by dot blot hybridization for the presence of a sequence homologous to that of *drb* with a conserved DNA probe derived from the *afa-1* operon (probe A in Fig. 1). Strains positive by dot blot hybridization were confirmed by Southern hybridization using the same probe. Sequence homology to *drb* was found in 49 of the 315 first UTI strains (16%), 7 of the 49 second UTI strains (14%), 2 of the 28 RUTI strains (7.1%), and 20 of the 395 fecal strains (5%); 3.6% of the 166 fecal strains from women with previous UTI history and 6.1% of the 229 fecal strains from women without a UTI history were *drb* positive ($P < 0.001$). Hybridization with the *drb* gene probe was 3.5 (odds ratio) (95% confidence interval, 2.0 to 5.9) times more common among *E. coli* isolates from patients with first cystitis than from control fecal isolates.

Adhesin subtype classification. Because of the homology between the adhesin subtypes, DNA hybridization cannot distinguish one subtype from another. We used PCR to differentiate between subtypes for each *drb*-positive *E. coli* isolate. Each known subtype was identified by PCR using a pair of primers: a conserved primer derived from the sequence immediately upstream of the adhesin-encoding gene that was conserved among all subtypes, and type primers derived from various regions of the E gene sequence where each given subtype had a sequence uniquely different from those of all other subtypes (Table 1). However, there were two exceptions: since *afaE3* and *draE* are 99.4% identical, and *afaE5* and *f131E* are 97% identical, at the DNA level, the same type primer was used within each pair. AfaE-III and Dr, and AfaE-V and F131, were thus classified into the same groups by PCR. We confirmed that the correct subtype was amplified in each PCR by showing the different-size PCR fragments were produced by the different types of primers (353 to 487 bp [Table 1]), a result verified by agarose gel electrophoresis (Fig. 2).

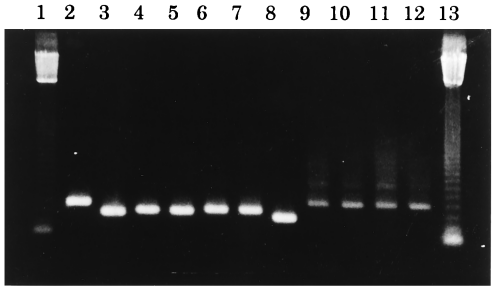

FIG. 2. Agarose gel analysis of PCR amplification of E gene regions from *E. coli* strains containing *drb* sequences. Lanes 1 and 13, 123-bp DNA ladder (GIBCO); lane 2, KS52 (AfaE-I); lane 3, A22 (AfaE-II); lane 4, A22 (AfaE-III); lane 5, H111S (Dr); lane 6, AL851 (AfaE-V); lane 7, C131 (F131); lane 8, C1845 (F1845); lane 9, 1154-11 (Drb122); lane 10, 9051-11 (Drb122); lane 11, F52-56 (Drb122); lane 12, F56-62 (Drb121).

Discovery of *afaE3-draE* and *draE-afaE3* hybrids. To differentiate between AfaE-III and Dr hemagglutinin as well as between AfaE-V and F131, we used the minor changes in endonuclease restriction sites caused by the difference between the respective pairs of sequences. A 534-bp DNA fragment containing the entire E gene from all four subtypes was PCR amplified. Of the PCR-amplified DNA fragments, AfaE-V was distinct from F131 by a *Bam*HI restriction site (none in AfaE-V and one in F131) and by *Ava*II restriction sites (two in AfaE-V and one in F131). AfaE-III was distinct from Dr hemagglutinin by *Tsp509*I restriction sites (one in AfaE-III and two in Dr hemagglutinin) and by a *Hsp92*I restriction site (none in AfaE-III and one in Dr hemagglutinin). By the restriction pattern, the F131–AfaE-V group could be clearly broken down into two expected subtypes: 4 F131 types and 32 AfaE-V types. This was not true for the AfaE-III–Dr hemagglutinin group; of 19 strains, 1 and 9 could be classified as AfaE-III and Dr, respectively, by both restriction sites. Eight strains were Dr by *Tsp509*I restriction pattern but AfaE-III by *Hsp92*I restriction pattern (*afaE3-draE* hybrids). One was AfaE-III by *Tsp509*I restriction pattern but Dr by *Hsp92*I restriction pattern (*draE-afaE3* hybrid).

One representative strain from each of the *afaE3-draE* and *draE-afaE3* hybrid groups was chosen for sequence analysis. The three nucleotides that differentiate *afaE3* and *draE* are at positions +217 (an A in *afaE3* or a G in *draE*), +326 (T or C, respectively), and +395 (C or T, respectively). The sequenced *afaE3-draE* hybrid had the nucleotides A, C, and T at these three sites and also had one nucleotide different from either *afaE3* or *draE* at position +22. This difference is predicted to change one amino acid in the signal sequence from Ala to Thr. The sequenced *draE-afaE3* hybrid had two silent changes at positions +24 and +411. Since position +217 (amino acid 52 in the mature adhesin) has been shown to be critical for both fimbriation and receptor binding characteristics (15), we predicted that *afaE3-draE* hybrids would be phenotypically AfaE-III-like and that the *draE-afaE3* hybrid would be phenotypically Dr-like. A hemagglutination assay (see Materials and Methods) was used to test for inhibition of MRHA by chloramphenicol. As predicted, the four hemagglutination-positive *afaE3-draE* hybrids were resistant to inhibition by chloramphenicol (similar to *afaE3*-positive strains), while the *draE-afaE3* hybrid had its hemagglutination inhibited by chloramphenicol (similar to *draE*-positive strains).

TABLE 2. Percent *drb* subtype distribution among all strains screened by strain source

Group (no. of isolates)	No. (%)								
	AfaE-I	F1845	AfaE-II	AfaE-III-like[a]	Dr-like[b]	AfaE-V	F131	Nontypeable	All types
1st UTI (315)	8 (2.5)	1 (0.3)	1 (0.3)	5 (1.6)	7 (2.2)	20 (6.3)	4 (1.3)	3 (1.0)	49 (16)
Fecal (395)	4 (1.0)	0 (0)	0 (0)	3 (0.8)	2 (0.5)	8 (2.0)	0 (0)	3 (0.7)	20 (5.1)
2nd UTI (49)	2 (4.1)	0 (0)	0 (0)	1 (2.0)	1 (2.0)	3 (6.1)	0 (0)	0 (0)	7 (14)
RUTI (28)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)	0 (0)	1 (3.6)	2 (7.1)
All sources (787)	14 (1.8)	1 (0.1)	1 (0.1)	9 (1.1)	10 (1.3)	32 (4.1)	4 (0.5)	7 (0.9)	78 (9.9)

[a] Includes eight *afaE3-draE* hybrids that are predicted to have phenotypes similar to that of AfaE-III (see the text).
[b] Includes one *draE-afaE3* hybrid that is predicated to have a Dr phenotype (see the text).

Subtype distribution by strain source. Every known Dr adhesin subtype was found in our urinary *E. coli* collection, but the subtypes occurred at different frequencies. Among the *drb*-positive UTI strains, 41% were AfaE-V, 18% were the AfaE-I subtype, 13% were Dr-like (nine *draE* plus one *draE-afaE3* hybrid), 12% were AfaE-III-like (one *afaE3* plus eight *afaE3-draE* hybrids), 5% were F131, 1% were AfaE-II, and 1% were the F1845 subtype. While the predominant subtypes were the same in both UTI and fecal isolates, the relative frequencies were very different (Table 2). Every subtype was found more frequently among urinary than fecal isolates. The most common subtype, AfaE-V, was over three times more frequent in first UTI than fecal isolates ($P = 0.01$), as were the Dr-like subtypes ($P = 0.03$).

Cloning and sequencing of new adhesin-encoding genes. Seven of the 78 total *drb* probe-positive strains (four UTI strains and three fecal strains) could not be classified as any known subtype by PCR. These strains could represent additional adhesin variants or deletions in the gene clusters. To address this question, we used both PCR cloning and genomic cloning to identify potential DNA segments that carry alternative or deleted E genes. The cloned DNA segments were sequenced to identify the relatedness to known Dr subtypes.

All known subtypes have a conserved DNA sequence immediately upstream of the E gene and share two conserved amino acids at the C-terminal end. Thus, we hypothesized that additional adhesin variants could have similar sequence conservation. A pair of primers was used to amplify the full length of the E gene region in the seven strains with a possible new adhesin subtype: the same conserved primer used in PCR typing and a downstream degenerate primer (Table 1) including all DNA sequences which could encode the conserved C-terminal amino acids and stop codon. Various amplification conditions were tried, and a DNA fragment with the expected size (~580 bp) was obtained from four of the seven strains. These DNA fragments were cloned and sequenced. Sequence results identified two new classes of E genes, *drb121* and *drb122*. The *drb121* gene gives a predicted amino acid sequence for Dr121 that matches most closely that of F1845 (48% identical, 69% similar), while *drb122* gives a predicted amino acid sequence for Dr122 that matches most closely those of F131 and AfaE-V (78% identical, 88% similar).

The remaining three isolates did not produce a band by PCR. Based on Southern hybridization and PCR analysis, these three strains differ in the 5′ flanking regions; thus, the conserved primer does not work in these three strains (data not shown). We then used genomic cloning to isolate the E gene from the three remaining strains. Total DNA was digested with *Sma*I. DNA fragments of about 3 and 3.5 kb, which should include the E gene region, were identified by Southern hybridization using an ~200-bp DNA probe that hybridized to sequences downstream of the *Sma*I site (Fig. 1). DNA fragments were purified from the agarose gel and cloned into the plasmid vector pZErO-1. Upon sequencing the cloned fragments, we identified two additional classes of E genes, which we named *nfa111* and *nfa116*. While Drb121 and Drb122 appear to belong to the previously defined Dr family, NFA111 and NFA116 have no amino acid sequence similarity with any Dr adhesin subtype. NFA111 and NFA116 do have sequences similar to the NFA series (Table 3). Although NFAs have not previously

TABLE 3. Pairwise percent identity[a] of deduced protein sequence among various *drb* subtypes

Subtype	% Identity[b]											
	AfaE-I	AfaE-II	AfaE-III	Dr[c]	F1845	AfaE-V	F131	Drb121	Drb122	NFAI	NFA111	NFA116
AfaE-I	100											
AfaE-II	27	100										
AfaE-III	37	31	100									
Dr[c]	37	31	98	100								
F1845	43	26	57	57	100							
AfaE-V	32	28	52	52	48	100						
F131	32	28	51	51	47	93	100					
Drb121	43	25	45	46	**48**	41	38	100				
Drb122	32	31	47	47	43	**78**	**78**	38	100			
NFAI[d]	—[e]	—	—	—	—	—	—	—	—	100		
NFA111	—	—	—	—	—	—	—	—	—	**38**	100	
NFA116	—	—	—	—	—	—	—	—	—	**98**	39	100

[a] As defined in Materials and Methods.
[b] Boldface numbers indicate the highest identity percentage between newly identified subtypes and a known subtype.
[c] DraE protein sequence used in comparisons.
[d] NfaA protein sequence (4) used in comparisons.
[e] —, Too different to obtain an identity comparison.

```
                PstI site
afa-1           CTGCAGTCA -GAAACTTACTTA -TATGCAATGAACA -GTCTCTGCTGCGGGTG
                CTGCAGTCA -GAAACTTACTTA -TATGCAATGAACA -GTCTCTGCTGCGGGTG
nfa116          CTGCAGTCAAGAAACTTACTTAATATGCAATGAACAAGTCTCTGCTGCGGGTG

afa-1           CAGACATCTGTGAACGGTGGTTAATGTGGGGTAAGACAGCTTACTGATTC
                CAGACATCTGTGAACGGTGGTTAATGTGGGGTAAGACAG -TTACTGATTC
nfa116          CAGACATCTGTGAACGGTGGTTAATGTGGGGTAAGACAGTTTACTGATTC

afa-1           TGGGATGAATTAGACCGTACTGTTGTGTTACCCCCTCACAAAACTGAATA
                -GGGATGAATTA----------ACA -AACTG--T
nfa116          TGGGATGGATTAACAGA----------ACACAACTGGCTT

                start codon of E gene
afa-1           GGTAATCCAT-------ATG
                G----TCCAT-------ATG
nfa116          G----TCCATAAGCAAAATG
```

FIG. 3. Nucleotide sequence comparison between upstream regions of *afaE-1* (28) and *nfaA* (4). Dashes represent missing equivalent bases (gaps), *Pst*I sites are underlined, and start codons are in boldface.

been considered part of the Dr family, they are very similar in genetic organization (1, 16). Since these NFA series can be detected by the same *drb* probe and have upstream flanking sequences similar to those of the Dr family (Fig. 3), they may well belong to the same group. However, it remains to be shown that they bind to decay-accelerating factor.

Genetic comparison of various adhesin carrying operons. Genetic conservation has been demonstrated with a common 4.1-kb internal fragment shared by all *afa*-related members (25). Here we extend the comparison to all E gene classes, including our four newly identified ones. We compared restriction fragment length polymorphism (RFLP) patterns among all adhesin classes by Southern hybridization using the accessory gene probe (probe B in Fig. 1, with a 4.1-kb DNA fragment composed of three *Pst*I fragments [2.6, 1.1, and 0.4 kb] from the *afa-1* operon) and the E gene probe (three PCR-amplified DNA fragments in Fig. 2, lanes 2, 4, and 6). All *drb* subtypes including the NFA series share a very conserved 4.1-kb (three *Pst*I fragments [2.6, 1.1, and 0.4 kb]) segment (Fig. 4), while E genes and their 3′ flanking regions are highly variable (Fig. 5).

It has been suggested that *afaD3* is the second adhesin gene in the *afa-3* operon (15). To determine if this sequence was also conserved among the D genes in other subtypes, we used PCR analysis with a pair of primers internal to the *afaD3* gene.

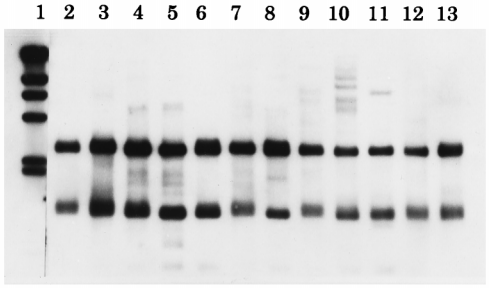

FIG. 4. Southern hybridization of total DNA from *drb*-positive strains representing each subtype, using a 4.1-kb DNA derived from the *afa-1* operon (probe B in Fig. 1) as the probe. DNAs were digested with *Pst*I. Lane 1, λ *Hin*dIII DNA marker; lane 2, K52 (AfaE-I); lane 3, 113-11 (AfaE-II); lane 4, 268-11 (AfaE-III); lane 5, H111S (Dr); lane 6, AL851 (AfaE-V); lane 7, C131 (F131); lane 8, C1845 (F1845); lane 9, 164-13 (NFA111); lane 10, 1069-11 (NFA116); lane 11, F56-62 (Drb121); lane 12, 1154-11 (Drb122); lane 13, 9051-11 (Drb122).

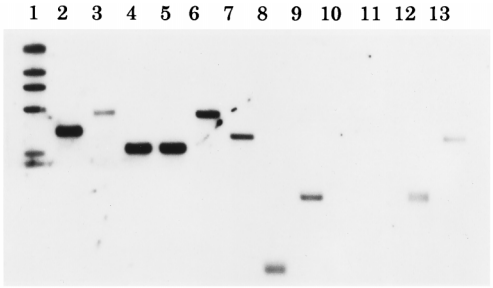

FIG. 5. Southern hybridization of total DNA from *drb*-positive strains representing each subtype, using three pooled PCR products (Table 1) representing AfaE-I, AfaE-III, and AfaE-V as the probe. DNAs were digested with *Pst*I. Lane 1, λ *Hin*dIII DNA marker; lane 2, K52 (AfaE-I); lane 3, 113-11 (AfaE-II); lane 4, 268-11 (AfaE-III); lane 5, H111S (Dr); lane 6, AL851 (AfaE-V); lane 7, C1845 (F1845); lane 8, F56-62 (Drb121); lane 9, F52-62 (Drb122); lane 10, 164-13 (NFA111); lane 11, 1069-11 (NFA116); lane 12, 1154-11 (Drb122); lane 13, 9051-11 (Drb122).

The D gene sequence appears to be conserved in most *drb* subtypes strains tested (Fig. 6). NFA111 shows no PCR fragment, and Drb122 shows a PCR fragment with smaller size.

Virulence profiles of strains producing Dr family adhesins. All of our *E. coli* isolates were screened by DNA hybridization for the presence of nine virulence factors: type I (*fim*), Pap and Prs pili (*prf*), S fimbria (*sfa*), aerobactin (*aer*), group II capsule (*kpsMT*), outer membrane protease T (*ompT*), α-hemolysin (*hly*), cytotoxic necrotizing factor 1 (*cnf1*), and adhesins of the Dr family (*drb*) as previously described (12). This genotyping generated a virulence signature for each strain based on the presence or absence of these nine virulence factors. Most of the *drb*-positive strains were also positive for *aer* (83%). Only one strain in our collection that was *drb* positive was positive for the toxin *hly* or *cnf1*. The Dr adhesins occurred only rarely with other MRHA adhesins such as S fimbria and P pili: 99 and 80% of *drb*-positive strains were negative for *sfa* and *prf*, respectively. Of greatest interest, half (51.3%) of the *drb*-positive strains belonged to a single virulence signature: 100111001 (Table 4), positive for *fim*, *aer*, *kpsMT*, *ompT*, and *drb* and lacking the other genes tested. This virulence signature was present in 7.6% of first UTI isolates, 8.2% of second UTI isolates, and 2.8% of fecal isolates. We carried out RFLP analysis using variation in the *fim* locus on the 25 isolates with the most common virulence signature and the *afaE5* gene. Figure 7 shows the hybridization patterns seen for seven strains with this predominant pattern, plus two strains with a second pattern and a third with yet a different pattern. In total, four

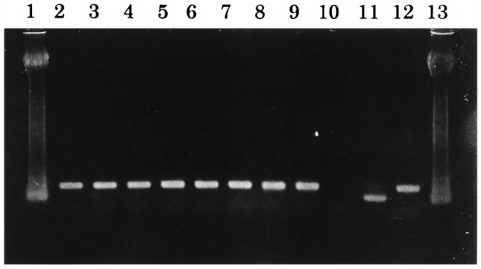

FIG. 6. Agarose gel analysis of PCR amplification of *afaD3*-related sequences in all *drb* member strains. Lanes 1 and 13, 123-bp DNA ladder (GIBCO); lane 2, C248 (AfaE-I); lane 3, 113-11 (AfaE-II); lane 4, 268-11 (AfaE-III); lane 5, H111S (Dr); lane 6, AL851 (AfaE-V); lane 7, C131 (F131); lane 8, C1845 (F1845); lane 9, 1069-11 (NFA116); lane 10, 164-13 (NFA111); lane 11, F52-62 (Drb122); lane 12, F56-62 (Drb121).

TABLE 4. Distribution of virulence signatures for *drb* subtype-carrying urinary and fecal *E. coli* isolates

Signature[a]	Distribution								
	AfaE-I	AfaE-II	AfaE-III-like	Dr-like	AfaE-V	F131	F1845	Others	Total
100000001	1				1				2
100001001	1								1
100010001					1	1		1	3
100011001			1						1
100100001				1	3			2	6
100101001			1						1
100110001			1				1	2	4
100111001	1		3	8	25	3			40
110000001	4								4
110001001	1								1
110010101					1				1
110100001			2						2
110110001	4							1	5
110111001		1	1	1				1	4
110111101	2								2
111111111					1				1

[a] 1 in a signature pattern indicates presence of factor. From left to right, factors are *fim*, *prf*, *sfa*, *aer*, *kpsMT*, *ompT*, *hly*, *cnf1*, and *drb*.

different patterns were present, 20 like that in lanes 2 through 8 of Fig. 7, three like those in lanes 9 and 10, one shown in lane 11, and one not pictured in Fig. 7.

Chromosomal and plasmid location of *drb* genes. The presence of the Dr family of adhesins is highly correlated with the presence of aerobactin (encoded by *aer*). Since *aer* can also be plasmid borne, the correlation might be caused by physical linkage. To address this question, the locations of both *drb* and *aer* determinants among *drb*-positive strains were analyzed by Southern hybridization using DNA probes (Fig. 8). Only 12% of the *drb* genes were found on large plasmids; all of the remaining *drb* genes were located on the chromosome. By subtype, 30% of *draE*, 16% of *afaE5*, 11% of *afaE3*, and 0% of all other subtypes were found on plasmids. Of the 65 *drb*-positive strains that were also positive for *aer*, at most five (6%) had both genes on the same plasmid. Thus, the correlation between *drb* and *aer* genes can be only partially explained by colocalization on a plasmid.

DISCUSSION

Previous studies have failed to agree on the frequencies of *drb* in UTI and fecal *E. coli* isolates (2, 3, 8, 25, 33, 35). Several studies have small sample sizes and/or variable or poorly defined study groups probably contributing to the discrepancies. Frequencies may also differ by age, geographic location, or disease severity. Data pooled across several studies suggests that Dr family adhesins occur twice as frequently among UTI compared to fecal isolates (10). We observed a threefold difference in sequences homologous to *drb* when we compared the frequency among 315 first-time UTI isolates from otherwise healthy college women to the frequency among 395 fecal isolates from healthy college women without UTI (15% versus 5%). This finding strongly supports an association between *drb* and first-time UTI in otherwise healthy young women. AfaE-V was the most common subtype in each *E. coli* collection but occurred proportionally more frequently in first-time UTI than fecal isolates, suggesting a possible specific role in adherence to the bladder.

In subtyping our collection of *E. coli* strains, we discovered genes with a sequence intermediate between the sequence found in subtypes *afaE3* and *draE*. These intermediate strains were discovered when restriction enzyme analysis of the PCR products revealed that only 10 of 19 strains amplified with the subtype AfaE-III/Dr-type primer (Table 1) gave PCR products which corresponded to either *afaE3* or *draE* sequences. Eight products appear to be a hybrid containing the N-terminal half of *afaE3* and the C-terminal half of *draE*. One product is the opposite hybrid: it contains the N-terminal half of *draE* and the C-terminal half of *afaE3*. N-terminal regions are critical for the differences seen between AfaE-III and Dr relative to fimbrial status (15) and chloramphenicol sensitivity for hemagglutination (28, 36). Thus, our eight *afaE3-draE* hybrids should be phenotypically similar to AfaE-III in being nonfimbriated and chloramphenicol resistant, while the *draE-afaE3* hybrid should behave like Dr in being fimbriated and chloramphenicol sensitive. Since the phenotypic differences are the ones that we expect to play a role in any pathogenic differences, we did our distribution analysis with the *afaE3-draE* hybrids listed as AfaE-III-like and the *draE-afa3* hybrid listed as Dr-like in Tables 2 and 4.

The *drb* sequences in our *E. coli* collection included the whole spectrum of Dr subtypes previously identified as well as our four completely new ones, for a total of at least 10 E gene subtypes (depending on how *drbE* genes with intermediate sequence between *afaE3* and *draE* are categorized). Despite significant sequence differences among E genes, all previously identified Dr subtypes and as well as our newly identified Dr subtypes (including NFA-like sequences) share highly conserved genes within the same gene cluster. They have the same

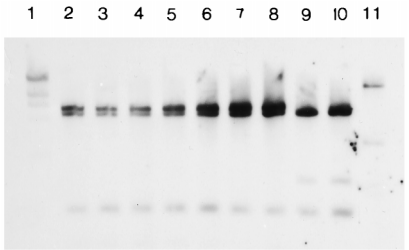

FIG. 7. Southern hybridization of total DNA from 10 of the 25 strains with the 100111001 virulence signature, using *fim* as the probe. DNAs were digested with *Hin*dIII and *Eco*RV. Lane 1, λ *Hin*dIII DNA marker; lane 2, 144-11; lane 3, 155-11; lane 4, 164-11; lane 5, 275-11; lane 6, 339-11; lane 7, 356-11; lane 8, F115-61; lane 9, 1188-11; lane 10, F1-62; lane 11, F200-61.

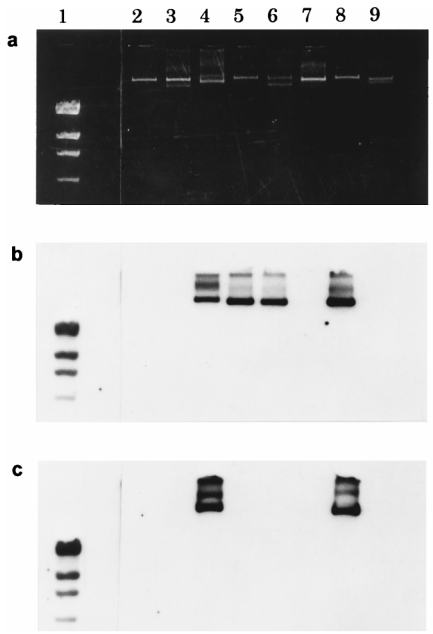

FIG. 8. (a) Agarose gel electrophoresis of purified plasmid DNAs. Lane 1, λ *Hin*dIII DNA marker; lane 2, 245-11 (AfaE-III); lane 3, 268-11 (AfaE-III); lane 4, 1175-11 (AfaE-III); lane 5, 1157-11 (AfaE-III); lane 6, 1072-11 (AfaE-V); lane 7, F1-62 (AfaE-V); lane 8, F34-62 (AfaE-V); lane 9, 365-11. (b) Southern hybridization of plasmid DNAs by using *drb* probe A (Fig. 1). (c) Southern hybridization of plasmid DNAs by using an *aer* probe.

DNA *Pst*I fragment of about 4.1 kb which has been demonstrated among *afa* operons (25). The ~150-bp segments between *Pst*I (p1 to p3 in Fig. 1) and the beginning of the E gene are almost identical among the classical Dr subtypes. Recent studies suggest that *afaD3* of the *afa-3* gene cluster may act as a second adhesin molecule (15). We found the D gene to be highly conserved.

Over half of the *drb*-positive strains in our collection had the same virulence signature: 100111001, which is positive for *fim*, *aer*, *kpsMT*, *ompT*, and *drb* and negative for *prf*, *sfa*, *hly*, and *cnf1* (Table 4). This virulence signature group accounted for 78% of the most common Dr subtype, AfaE-V, 80% of the Dr-like strains, and 75% of the F131 strains. Whether virulence signature 100111001 corresponds to serotype O75, which has been associated with the Dr family of adhesins (33, 39), is unknown.

There are at least three possible explanations for the observed association of the Dr adhesin with a set of virulence genes: (i) physical co-localization, (ii) clonality, and (3) codependence in particular pathogenic disease pathways. Pathogenicity islands containing multiple virulence factors such as P pili, hemolysin, and CNF1 found in some uropathogenic *E. coli* isolates (7, 37) are a prime example of physical colocalization. *fim*, *kpsMT*, *ompT*, and, when on the chromosome, *aer* and *drb* map to different chromosomal locations. Further, only 9% of *drb* genes in our strains are on plasmids, and only half of those plasmids also contain *aer*. Therefore colocalization of the genes does not seem to be a plausible explanation of the association of *drb* with this particular virulence signature.

If a single clone were responsible for a significant proportion of UTIs caused by Dr-adherent strains, then the copresence of the other virulence genes might be a chance occurrence. However, the majority of isolates from three different *drb* subtypes have the same virulence signature. RFLP analysis using variation in the *fim* locus showed that 20 of 25 isolates with the most common virulence signature and the *afaE5* gene fall into a single RFLP group. Therefore, half of the 40 strains with the predominant virulence signature are part of a single dominant clone, but the other half fit into at least eight other clonal types with no more than four members each, based on subtype and RFLP analysis.

Finally, codependence of these virulence factors in particular pathogenic disease pathways may explain the association of *drb* with a particular virulence signature. Our data support the possibility that strains with the 100111001 virulence signature use a particular pathogenic pathway to cause UTI. This pathway may differ from that used by UTI *E. coli* expressing P pili, hemolysin, and CNF1. Different pathogenic pathways may involve differences in mode of transmission. Epidemiologic studies exploring the latter possibility are ongoing.

Dr is associated with risk of a second UTI (13); thus, a better understanding of the role of Dr subtypes in pathogenesis is warranted. In a large population-based sample of UTI isolates, we found a particular Dr-associated virulence profile to predominate, suggesting an association of this profile with UTI pathogenesis. In between 20 and 30% of all women with a first UTI, the infection recurs within 6 months (11); recurrences account for the vast majority of UTIs treated annually. It will therefore be important to determine if the predominate Dr-associated virulence profile found in this study is also present in significant numbers of strains from women with recurrences.

ACKNOWLEDGMENTS

We thank Steve L. Moseley (University of Washington) for kindly providing unpublished F131 sequences; Charlotte Williams and other staff at the University of Michigan Student Health Service and Scott Spear, Shirley Arldt, Nell Curtis, Barbara Locke, and other staff at the University of Texas at Austin Student Health Service for bacterial samples; and Bonnie Andree, M. Joan Marty, Angela Spindler, and Divya Agrawal for assistance in data collection, data management, and storage and processing of study isolates.

This work was supported by Public Health Service grants DK-35368 and DK-47519.

REFERENCES

1. **Ahrens, R., M. Ott, A. Ritter, H. Hoschutzky, T. Buhler, F. Lottspeich, G. J. Boulnois, K. Jann, and J. Hacker.** 1993. Genetic analysis of the gene cluster encoding nonfimbrial adhesin I from an *Escherichia coli* uropathogen. Infect. Immun. **61:**2505–2512.
2. **Archambaud, M., P. Courcoux, and A. Labigne-Roussel.** 1988. Detection by molecular hybridization of *pap*, *afa*, and *sfa* adherence systems in *Escherichia coli* strains associated with urinary and enteral infections. Ann. Inst. Pasteur Microbiol. **139:**557–573.
3. **Arthur, M., C. E. Johnson, R. H. Rubin, R. D. Arbeit, C. Campanelli, C. Kim, S. Steinbach, M. Agarwal, R. Wilkinson, and R. Goldstein.** 1989. Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. Infect. Immun. **57:**303–313.
4. **Aubel, D., A. Darfeuille-Michaud, C. Martin, and B. Joly.** 1992. Nucleotide sequence of the *nfaA* gene encoding the antigen 8786 adhesive factor of enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **98:**277–284.
5. **Bilge, S. S., J. M. Apostol, Jr., K. J. Fullner, and S. L. Moseley.** 1993. Transcriptional organization of the F1845 fimbrial adhesin determinant of *Escherichia coli*. Mol. Microbiol. **7:**993–1006.
6. **Bilge, S. S., C. R. Clausen, W. Lau, and S. L. Moseley.** 1989. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J. Bacteriol. **171:**4281–4289.
7. **Blum, G., M. Ott, A. Lischewski, A. Ritter, H. Imrich, H. Tschape, and J. Hacker.** 1994. Excision of large DNA regions termed pathogenicity islands from tRNA-specific loci in the chromosome of an *Escherichia coli* wild-type pathogen. Infect. Immun. **62:**606–614.

7b. **Carnoy, C., and S. L. Moseley.** 1997. Mutational analysis of receptor binding mediated by the Dr family of *Escherichia coli* adhesins. Mol. Microbiol. **23:**365–379.

8. **Daigle, F., J. Harel, J. M. Fairbrother, and P. Lebel.** 1994. Expression and detection of pap-, sfa-, and afa-encoded fimbrial adhesin systems among uropathogenic *Escherichia coli*. Can. J. Microbiol. **40:**286–291.

9. **De Graaf, F. K., and F. R. Mooi.** 1986. The fimbrial adhesins of *Escherichia coli*. Adv. Microb. Physiol. **28:**74–143.
10. **Donnenberg, M. S., and R. A. Welch.** 1996. Virulence determinants of uropathogenic *Escherichia coli*, p. 135–174. *In* L. T. Mobley and J. W. Warren (ed.), Urinary tract infection: molecular pathogenesis and clinical management. American Society for Microbiology, Washington, D.C.
11. **Foxman, B.** 1990. Recurring urinary tract infection: incidence and risk factors. Am. J. Public Health **80:**331–333.
12. **Foxman, B., L. Zhang, K. Palin, P. Tallman, and C. F. Marrs.** 1995. Bacterial virulence characteristics of Escherichia isolates from first-time urinary tract infection. J. Infect. Dis. **171:**1514–1521.
13. **Foxman, B., L. Zhang, P. Tallman, K. Palin, C. Rode, C. Bloch, B. Gillespie, and C. F. Marrs.** 1995. Virulence characteristics of *Escherichia coli* causing first urinary tract infection predict risk of second infection. J. Infect. Dis. **172:**1536–1541.
14. **Garcia, M. I., P. Courcoux, A. Labigne, H. Ohayon, P. Gounon, and C. Le Bouguenec.** 1995. Adhesion of diffusely-adhering *Escherichia coli* producing the afimbrial adhesins AFA-III and AFA-V, abstr. B-10, p. 167. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
15. **Garcia, M. I., P. Gounon, P. Courcoux, A. Labigne, and C. Le Bouguenec.** 1996. The afimbrial adhesive sheath encoded by the *afa-3* gene cluster of pathogenic *Escherichia coli* is composed of two adhesins. Mol. Microbiol. **19:**683–693.
16. **Garcia, M. I., A. Labigne, and C. Le Bouguenec.** 1994. Nucleotide sequence of the afimbrial-adhesin-encoding *afa-3* gene cluster and its translocation via flanking IS*1* insertion sequences. J. Bacteriol. **176:**7601–7613.
17. **Goldhar, J., R. Perry, J. R. Golecki, H. Hoschutzky, B. Jann, and K. Jann.** 1987. Nonfimbrial, mannose-resistant adhesins from uropathogenic *Escherichia coli* O83:K1:H4 and O14:K?:H11. Infect. Immun. **55:**1837–1842.
18. **Goluszko, P., S. Nowicki, A. Kaul, T. Pham, S. Moseley, and B. Nowicki.** 1995. Development of experimental chronic pyelonephritis with Dr fimbriae bearing *Escherichia coli* O75:K5:H−, abstr. B-15, p. 168. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
19. **Hales, B. A., H. Beverley-Clarke, N. J. High, K. Jann, R. Perry, J. Goldhar, and G. J. Boulnois.** 1988. Molecular cloning and characterization of the genes for a non-fimbrial adhesin from *Escherichia coli*. Microb. Pathog. **5:**9–17.
20. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33:**933–938.
21. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9:**1989–1998.
22. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
23. **Kado, C. I., and S.-T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
24. **Krogfelt, K. A.** 1991. Bacterial adhesin: genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of *Escherichia coli*. Rev. Infect. Dis. **13:** 721–735.
25. **Labigne-Roussel, A., and S. Falkow.** 1988. Distribution and degree of heterogeneity of the afimbrial-adhesin-encoding operon (*afa*) among uropathogenic *Escherichia coli* isolates. Infect. Immun. **56:**640–648.
26. **Labigne-Roussel, A., M. A. Schmidt, W. Walz, and S. Falkow.** 1985. Genetic organization of the afimbrial adhesin operon and nucleotide sequence from a uropathogenic *Escherichia coli* gene encoding an afimbrial adhesin. J. Bacteriol. **163:**1285–1292.
27. **Le Bouguenec, C.** Unpublished results.
28. **Le Bouguenec, C., M. I. Garcia, V. Ouin, J. M. Desperrier, P. Gounon, and A. Labigne.** 1993. Characterization of plasmid-borne *afa-3* gene clusters encoding afimbrial adhesins expressed by *Escherichia coli* strains associated with intestinal or urinary tract infections. Infect. Immun. **61:**5106–5114.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Moseley, S. L.** Personal communication.
31. **Nowicki, B., A. Hart, K. E. Coyne, D. M. Lublin, and S. Nowicki.** 1993. Short consensus repeat-3 domain of recombinant decay-accelerating factor is recognized by *Escherichia coli* recombinant Dr adhesin in a model of a cell-cell interaction. J. Exp. Med. **178:**2115–2121.
32. **Nowicki, B., A. Labigne, S. Moseley, R. Hull, S. Hull, and J. Moulds.** 1990. The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. Infect. Immun. **58:**279–281.
33. **Nowicki, B., C. Svanborg-Eden, R. Hull, and S. Hull.** 1989. Molecular analysis and epidemiology of the Dr hemagglutinin of uropathogenic *Escherichia coli*. Infect. Immun. **57:**446–451.
34. **Savarino, S. J., P. Fox, D. Yikang, and J. P. Nataro.** 1994. Identification and characterization of a gene cluster mediating enteroaggregative *Escherichia coli* aggregative adherence fimbria I biogenesis. J. Bacteriol. **176:**4949–4957.
35. **Stapleton, A., S. Mosely, and W. E. Stamm.** 1991. Urovirulence determinants in *Escherichia coli* isolates causing first-episode and recurrent cystitis in women. J. Infect. Dis. **163:**773–779.
36. **Swanson, T. N., S. S. Bilge, B. Nowicki, and S. L. Moseley.** 1991. Molecular structure of the Dr adhesin: nucleotide sequence and mapping of receptor-binding domain by use of fusion constructs. Infect. Immun. **59:**261–268.
37. **Swenson, D. L., N. O. Bukanov, D. E. Berg, and R. A. Welch.** 1996. Two pathogenicity islands in uropathogenic *Escherichia coli* J96: cosmid cloning and sample sequencing. Infect. Immun. **64:**3736–3743.
38. **Westerlund, B., P. Kuusela, J. Risteli, L. Risteli, T. Vartio, H. Rauvala, R. Virkola, and T. K. Korhonen.** 1989. The O75X adhesin of uropathogenic *Escherichia coli* is a type IV collagen-binding protein. Mol. Microbiol. **3:**329–337.
39. **Yamamoto, S., T. Tsukamoto, A. Terai, H. Kurazono, Y. Takeda, and O. Yoshida.** 1995. Distribution of virulence factors in Escherichia coli isolated from urine of cystitis patients. Microbiol. Immunol. **39:**401–404.

Editor: P. E. Orndorff