

Virulence Properties and Clonal Structures of Strains of *Escherichia coli* O119 Serotypes

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A total of 110 *Escherichia coli* strains of serogroup O119 were examined for the presence of virulence properties characteristic of enteropathogenic *E. coli* (EPEC). Three virulence patterns were distinguished based on the detection of a chromosomal gene mediating intimate attachment (*eaeA*) and plasmid DNA involved in localized adherence (EAF and *bfpA*). The first pattern, represented by strains which hybridized with three gene probes, was the most common (68%) and, with a single exception, included only O119:H6 strains. Of these strains, 90% showed a typical localized adherence (LA) pattern in HEp-2 cells and 96% were positive for intimate attachment in a fluorescent-actin staining test with a 3-h incubation period. The second pattern was represented by strains which hybridized with the *eaeA* gene only. Most (89.5%) of these strains showed the LA phenotype but only after 6 h of incubation (LA-like phenotype). The third pattern consisted of strains which were positive for *eaeA* and *bfpA* but did not hybridize with the EAF probe. Most (80%) of these strains exhibited the LA-like phenotype. Analysis of several *eaeA*⁺ *bfpA*⁺ strains for the expression of the pilin subunit (BfpA) of the bundle-forming pili demonstrated that all LA strains expressed BfpA whereas the LA-like strains did not. The study of the clonal relationships, carried out by multilocus enzyme electrophoresis in 79 representative strains, defined 11 distinct electrophoretic types (ETs). ET1 included 66% of the strains, most of which displayed the *eaeA*⁺ *bfpA*⁺ EAF⁺ pattern and were serotyped as O119:H6 or O119:H⁻. The remaining 10 ETs were each represented by no more than five strains and, with the exception of ET8, included strains of a single serotype. The genetic relatedness of the ETs revealed two main clusters, with most strains in cluster A having the *eaeA*⁺ *bfpA*⁺ EAF⁺ combination and a O119:H6 serotype. Cluster B was represented by atypical EPEC strains with only the *eaeA*⁺ and the *eaeA*⁺ *bfpA*⁺ virulence pattern.

Enteropathogenic *Escherichia coli* (EPEC) was first implicated in human infectious disease in the 1940s as a result of conspicuous outbreaks of acute infantile gastroenteritis (24, 35). EPEC strains infect human populations throughout the world and remain one of the primary causes of infantile diarrhea in developing countries (1, 7, 12). EPEC strains have been incriminated in outbreaks of disease and also are common in endemic (sporadic) cases. In Brazil, for example, EPEC strains are isolated from 30% or more of infants of low socioeconomic status with diarrhea (13, 44, 45).

Historically, EPEC strains have been identified by serotype, i.e., the distinct combination of O (somatic) and H (flagellar) antigens, which have been linked epidemiologically to infantile diarrhea. In 1987, the World Health Organization (49) recognized EPEC serotypes of 12 different O serogroups (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158). In the past 15 years, however, the tools for identifying EPEC have been refined as the molecular basis of EPEC pathogenesis has begun to be elucidated and specific virulence genes have been discovered (18).

Recently, our laboratories have collaborated to investigate the virulence properties that define EPEC strains. We have used protein polymorphisms detected by multilocus enzyme

electrophoresis to resolve the major bacterial clones within EPEC serogroups and to build a population genetic framework for understanding the relationship between serotype and the virulence properties of strains. Clonal analysis of *E. coli* of serogroups O114 (3), 126 (50), O111 (6) and O55 (36) has shown that these EPEC serogroups are diverse mixtures of bacteria, often composed of a variety of clones that have acquired distinct combinations of virulence factors, both plasmid borne and chromosomal, and that these combinations correspond to distinct modes of pathogenesis.

The purpose of this study was to analyze virulence properties and the clonal relationships of *E. coli* strains of the O119 serogroup which have been found in association with infantile diarrhea in many countries (10, 13, 45). In Brazil, for example, O119 strains comprise the second most frequently detected EPEC serogroup associated with endemic infantile diarrhea (13, 45). In the United States, O119 strains have been implicated in outbreaks of acute diarrhea (30, 37), and in Thailand, O119 seems to be the most frequently serogroup isolated from children with diarrhea (8). Although epidemiologically significant, the virulence properties and genetic relatedness of strains of this serogroup have not been investigated.

MATERIALS AND METHODS

Bacterial strains. A total of 110 strains of *E. coli* serogroup O119 from different countries were studied. The strains were initially identified by serological methods (9), and after reisolation in MacConkey agar, the O and H antigens were confirmed. The origin, period of isolation, and serotype of these strains are shown in Table 1.

Gene probes. All the strains were tested for the presence of 13 different genes or gene regions associated with virulence, i.e., toxin production or adhesion

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TABLE 1. Sources and flagellar antigens (H types) of 110 *E. coli* O119 strains

Origin	Period of isolation	No. of strains	No. of the H type:				
			H ⁻	H2	H6	H7	H18
Brazil							
São Paulo	1960–1993	61	4	0	56	0	1
São Paulo	Unknown	2	0	0	2	0	0
Ribeirão Preto	1993	19	0	3	16	0	0
Chile	Unknown	4	0	0	4	0	0
Denmark	1960	1	0	1	0	0	0
United States							
CDC ^a	1960–1985	17	1	1	14	1	0
St. Louis	Unknown	1	0	0	1	0	0
Unknown	Unknown	1	0	0	1	0	0
Mexico	Unknown	4	0	1	3	0	0
Total		110	5	6	97	1	1

^a CDC, Centers for Disease Control and Prevention.

expression. The following specific gene probes were used: AA (enteroaggregative *E. coli* adherence plasmid), a 1-kb *EcoRI-PstI* fragment of pCVD432 (2); *bfpA* (BfpA structural gene), an 852-bp *EcoRI* fragment of pMSD207 (11); EAF (EPEC adherence factor), a 1-kb *BamHI-Sall* fragment derived from plasmid pMAR2 (31); *eaeA* (*E. coli* attaching-and-effacing gene encoding intimin) (16), a 1-kb *Sall-KpnI* fragment from plasmid pCVD434; *daaC* (a gene whose product is associated with the assembly of the F1845 fimbria of diffusely adherent *E. coli* strains), a 350-bp *PstI* fragment of pSLM852 (5); *stx₁* (Shiga-like toxin I gene), a 1,142-bp *BamHI* fragment of pJN37-19 (32); *stx₂* (Shiga-like toxin II gene), an 842-bp *PstI* fragment from plasmid pNN111-19 (32); EHEC plasmid, a 3.4-kb *HindIII* fragment of pCVD419 (26); INV (*E. coli* invasiveness factor), a 2.5-kb *HindIII* fragment from pSF55 (41); LTI (*E. coli* heat-labile enterotoxin type I), a 1.2-kb *HincII* fragment from pEWD299 (29); LTII (*E. coli* heat-labile enterotoxin type II), an 800-bp *HindIII-PstI* fragment from pCP2725 (33); STH (*E. coli* heat-stable enterotoxin of human origin), a 215-bp *HpaII* fragment obtained from digestion of a *HindIII-EcoRI* fragment from pSLM004 (28); and ST-I (p) (*E. coli* heat-stable enterotoxin of porcine origin), a 157-bp *HinfI* fragment of pRIT10036 (29). The occurrence of the *bfpA* and EAF sequences in the EAF plasmid was demonstrated by Southern blot. DNA probe fragments were labeled with dATP by nick translation (34) and used in colony hybridization (27).

Adherence properties. The pattern of bacterial attachment to HEp-2 cells in the presence of D-mannose was assayed by previously described methods (38). Monolayers were examined after 3 and 6 h of incubation.

FAS test. Strains were examined by the fluorescent-actin staining (FAS) test to detect filamentous actin in tissue culture cells polymerized beneath intimately attached bacteria (20, 21). The strains were tested after both 3 and 6 h of incubation as described previously (6, 36). Strains promoting dense actin concentrations were classified as FAS⁺, and those showing no dense actin concentrations were classified as FAS⁻.

E-Hly. Enterohemolysin (E-Hly) production was detected by the method described by Beutin et al. (4). Strains were considered positive for E-Hly when halos of hemolysis were observed around colonies only after 24 h of incubation.

Production of BfpA. To determine if bacteria produced the bundle-forming pilus structural subunit (BfpA), strains were grown overnight at 37°C in 3 ml of minimal essential medium supplemented with 10% fetal bovine serum. The cultures were centrifuged at 12,000 × g for 5 min, and the pellets were suspended in 100 μl of phosphate-buffered saline (pH 7.4) plus 20 μl of sample buffer (20% sodium dodecyl sulfate [SDS], 20% glycerol, 20 mM Trizma base [pH 6.8], 0.01% bromophenol blue). Protein extracts (10 μg per well) were electrophoresed in an SDS-polyacrylamide gel electrophoresis gel (16% polyacrylamide) as described by Laemmli (23) in Tris-glycine buffer (50 mM Trizma base; 384 mM glycine, 0.1% SDS). Proteins were transferred to a nitrocellulose membrane by Western blotting as described by Harlow and Lane (15). The nitrocellulose filter was blocked for 2 h (phosphate-buffered saline [pH 7.4], 0.5% Tween 20, 3% skim milk) at room temperature, washed, and incubated with anti-BfpA polyclonal antiserum (1:2,000) (kindly provided by Jorge A. Girón). Anti-immunoglobulin G conjugated with peroxidase (Sigma) was added (1:2,000), and the blot was developed with 10 mg of 3,3'-diaminobenzidine tetrahydrochloride (Sigma) in Tris-HCl (pH 7.6)-H₂O₂ (Sigma).

Multilocus enzyme electrophoresis. To analyze enzyme electrophoretic variation, bacterial cell lysates were prepared and subjected to multilocus enzyme electrophoresis (40). Twenty enzymes (see Table 4) were examined for allelic variation as described previously (48). Genetic relationships among strains were estimated by equating electromorphs with alleles at the corresponding enzyme locus and designating distinctive multilocus genotypes as electrophoretic types (ETs). Genetic distances were calculated and used to generate a dendrogram by the neighbor-joining algorithm (22). The statistical significance of clusters in the

dendrogram was assessed by bootstrapping as described previously (48). For this study, we selected a representative number of O119:H6 and O119:H⁻ strains and all the strains of the remaining serotypes.

RESULTS

Virulence properties of serotypes. Table 2 shows the combination of virulence genes and the phenotypic characteristics of the strains of each serotype. Serotypes O119:H6 and O119:H⁻ were represented by strains with three patterns of virulence genes (*eaeA*⁺ *bfpA*⁺ EAF⁺, *eaeA*⁺ *bfpA*⁺, and *eaeA*⁺), and serotypes H2 and H7 were represented by strains with the single *eaeA*⁺ *bfpA*⁺ combination. None of the 110 strains studied reacted with the probes for genes associated with aggregative or diffuse adherence (AA and *daaC*), the enterohemorrhagic *E. coli* adherence plasmid (EHEC), invasiveness (INV), or Shiga toxin and enterotoxins [Stx1, Stx2, LT-I, LT-II, ST-I (h), and ST-I (p)]. One single O119:H18 strain lacked the virulence genes found in strains of other serotypes.

Three distinct patterns of adherence were distinguished: LA, when bacteria bound to localized areas of the HEp-2 cells forming distinct microcolonies after 3 h of incubation (Fig. 1A); LA-like (LAL), in which bacteria formed microcolonies less dense and compact than those displayed by typical LA strains, after 6 h of incubation (Fig. 1B); and an indefinite pattern, in which the adhesion was very light, involving only few bacterial cells (Fig. 1C) but giving a positive result in the FAS test. Most strains with the *eaeA*⁺ *bfpA*⁺ EAF⁺ combination exhibited the LA pattern, while the majority of the strains with either the *eaeA*⁺ *bfpA*⁺ combination or only the *eaeA* gene exhibited the LAL pattern. The indefinite pattern was shown by all LAL strains after 3 h of incubation and by five other strains only after 6 h. It is noteworthy that of the 74 strains with the *eaeA*⁺ *bfpA*⁺ EAF⁺ combination, only 7 did not have the LA phenotype: 4 expressed the LAL pattern, and 3 had an indefinite adherence.

Of the 90 *eaeA*⁺ *bfpA*⁺ EAF⁺ or *eaeA*⁺ *bfpA*⁺ strains, 85 (95%) were FAS⁺ in the 3-h assay whereas of the 19 *eaeA*⁺

TABLE 2. Virulence characteristics of the *E. coli* O119 serotypes

Sero-type	No. of strains	Virulence pattern ^a	No. of strains with adherence pattern to HEp-2 cells ^b :				No. of strains reacting in the FAS assay after:	
			LA	LAL	IN	NA	3 h	6 h
H6	97	<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ EAF ⁺ (74) ^c	67	4	3		71	2
		<i>eaeA</i> ⁺ (16)		15	1		9	7
		<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ (7)	2	5			5	2
H2	6	<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ (6)		6			6	
H ⁻	5	<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ EAF ⁺ (1)	1				1	
		<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ (1)	1				1	
		<i>eaeA</i> ⁺ (3) ^c		2	1		1	
H7	1	<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ (1)		1			1	
H18 ^d	1	None			1			

^a EAF, EPEC adherence factor; *bfpA*, gene encoding BfpA pilin subunit; *eaeA*, EPEC attaching-and-effacing intimin gene. The number of strains is given in parentheses.

^b LA, localized adherence (3 h of incubation); LAL, LA-like pattern (6 h of incubation); IN, indefinite adherence pattern (6 h of incubation); NA, not adherent.

^c Includes a strain(s) with inconclusive results in the FAS assay.

^d Negative in the FAS assay.

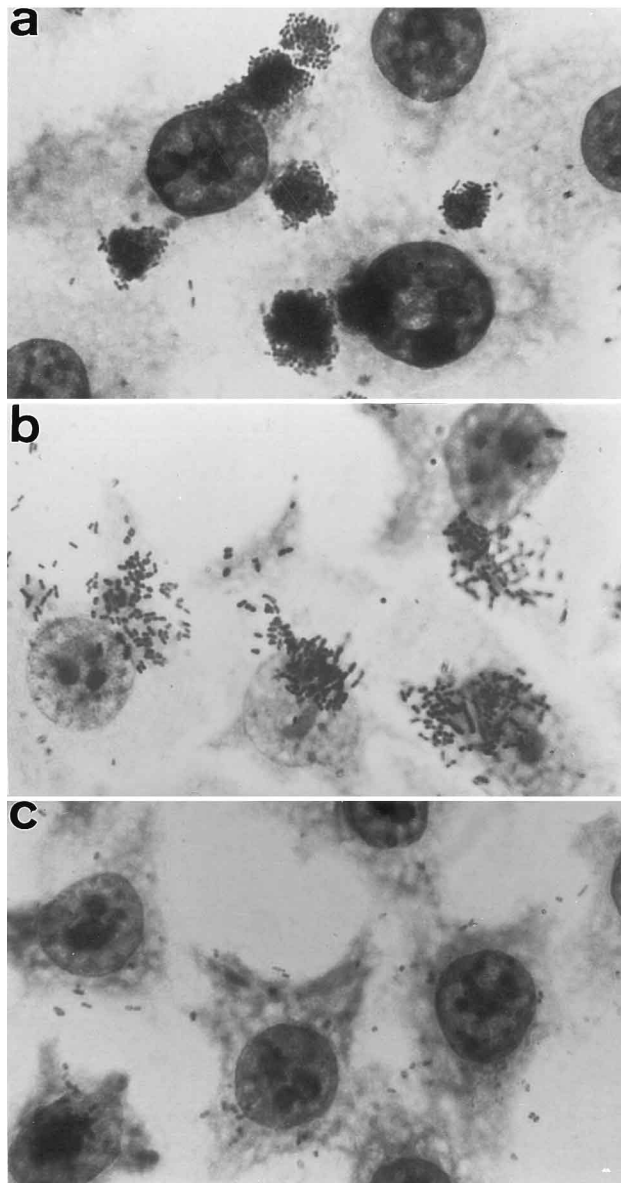


FIG. 1. Adherence patterns of O119 strains. (A) Localized adherence of an O119:H6 strain of cluster A with the *eaeA-bfpA*-EAF gene combination typical of EPEC (3 h of incubation). (B) LAL pattern of an O119:H2 strain harboring an incomplete EAF plasmid, that is, a plasmid lacking the EAF region (6 h of incubation). (C) Indefinite adherence pattern (6 h of incubation).

strains, only 10 were FAS⁺ (Table 2). Seven of the remaining *eaeA*⁺ strains were FAS⁺ after 6 h of incubation.

Production of E-Hly. Only 1 of the 110 strains tested was positive for E-Hly.

Production of BfpA. Of the 15 *eaeA*⁺ *bfpA*⁺ strains tested for BfpA production, 3 exhibited the LA pattern and 12 exhibited the LAL pattern. The 3 LA strains produced BfpA, but none of 12 LAL strains produced this fimbria.

Combination of virulence genes and period of isolation of the strains. Some virulence genes, especially those on plasmids, can be readily lost in culture and long-term storage (25). Table 3 shows that the *eaeA*⁺ and *eaeA*⁺ *bfpA*⁺ strains of serotype O119:H6 were isolated more frequently before 1981. In contrast, the *eaeA* *bfpA* strains of serotype O119:H2 and all the *eaeA*⁺ *bfpA*⁺ EAF⁺ strains were isolated more frequently after 1981.

Enzyme polymorphism and ETs. A total of 79 strains (67 O119:H6, 4 O119:H⁻, 6 O119:H2, 1 O119:H7, and 1 O119:H18) were examined for allelic variation at 20 enzyme loci by multilocus enzyme electrophoresis. Of the 20 enzyme-encoding loci, 12 were polymorphic, with an average of 2.3 alleles resolved per locus. Eleven ETs were identified, with 52 (65.8%) strains belonging to a single ET (ET1) (Table 4).

Genetic relationship of clones. Genetic distances among the 11 O119 ETs were estimated from the allele profiles (Table 4). The dendrogram (Fig. 2) illustrates the genetic similarity and degree of divergence between the 11 ETs and their relationship with serotypes. The 11 ETs were distributed in two main clusters, designated A and B (Fig. 2). Cluster A ETs were all closely related, with only minor differences in their enzyme allele profiles (Table 4). Cluster A includes only the O119:H6 strains and the nonmotile O119 strains. In fact, all the nonmotile strains were identical in enzyme profile to ET1, the most common ET, composed of motile strains with H6 antigen. Cluster B includes all of the H2 strains, two H6 strains, and one H7 strain. Cluster C was defined by a single isolate with serotype O119:H18.

There was a close correlation between clusters and virulence properties. Most (74%) of the strains in cluster A were positive for both the EAF region and *bfpA*, whereas all the cluster B strains were *bfpA*⁺ and were EAF⁻. Strains of both cluster A and cluster B carried the *eaeA* gene. The single strain of cluster C lacked all of these virulence properties. Because the cluster A O119 strains are so closely related, as indicated by their similarity in ET, and express the adhesion properties characteristic of EPEC, we infer that these ETs mark a widespread EPEC clonal group. Support for this hypothesis comes from the fact that cluster A includes O119:H6 strains implicated in epidemics of infantile diarrhea. One ET4 strain (CDC 277-84) of cluster A was originally isolated during an outbreak of acute infantile gastroenteritis in the United States in 1980 in which attachment-effacement was observed in tissue obtained during intestinal biopsies (37). A second cluster A strain (CDC 843-60, ET6) was originally recovered during an outbreak of acute infantile diarrhea in the United States in 1960 (30).

An association between ET and the origin of the strains was also observed for 9 of the 11 O119 ETs (Fig. 2). The single ET11 strain and all the ET1 and ET3 strains were isolated in Brazil; ET2 was isolated in Mexico; and ET4, ET5, ET6, ET9, and ET10 were isolated in the United States. However, ET7 and ET8 included strains from distinct locations. ET7 was represented by strains isolated in Mexico and Brazil, and ET8 included strains from Denmark, Mexico, and United States.

DISCUSSION

In this study, we have examined a diverse collection of *E. coli* O119 strains isolated from human disease in different coun-

TABLE 3. Distribution of 96 O119 strains with the different gene profiles according to the period of isolation

Period of isolation	No. of strains	No. of strains of ^a :			
		<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ EAF ⁺ [H6]	<i>eaeA</i> ⁺ <i>bfpA</i> ⁺ [H6]	H2	<i>eaeA</i> ⁺ [H6]
1960–1980	67	42 (63%)	6 (9%)	2 (3%)	17 (25%)
1981–1993	29	24 (83%)	1 (3.5%)	3 (10%)	1 (3.5%)
<i>P</i> ^b		0.041	0.316	0.160	0.002

^a Brackets indicate nonmotile strains of the same ET.

^b *P* values from Fisher's exact test.

TABLE 4. Eleven ETs defined by distinct allele combinations for 20 enzyme-encoding loci in strains of serogroup O119

ET	No. of isolates	No. of alleles at enzyme locus ^a :																			
		PGI	IDH	ACO	G3P	PE2	AK	MDH	PGD	M1P	GOT	BGA	ADH	MPI	G6P	IPO	CAK	NSP	TDH	SKD	GLU
1	52	6	3	4.2	4	4	4	4	6	3	4	5	6	4	4	4	4	2	4	7	2
2	2	6	3	4.2	4	4	4	4	6	3	4	6	6	4	4	4	4	2	4	7	2
3	2	6	5	4.2	4	4	4	4	6	3	4	5	0	4	4	4	4	2	4	7	2
4	3	6	5	4.2	4	4	4	4	6	2	4	4	6	4	4	4	4	2	0	6	2
5	5	6	5	4.2	4	4	4	4	6	2	4	6	6	4	4	4	4	2	4	6	2
6	5	6	3	4.2	4	4	4	4	6	2	4	6	6	4	4	4	4	2	4	6	2
7	4	6	5	7	4	5	2	4	6	6	6	2.5	6	10	4	4	4	2	4	4	2
8	3	6	5	7	4	5	2	4	6	7	6	2.5	6	10	4	4	4	2	4	4	2
9	1	6	5	7	1.5	5	2	4	6	7	6	2	0	0	4	4	4	2	0	4	2
10	1	6	5	6	4	5	2	4	6	8	6	0	4	8	4	4	4	2	4	4	2
11	1	6	5	6.2	4	4	2	4	6	8	6	2.5	6	10	4	4	4	2	4	6	2

^a Abbreviations: PGI, glucosephosphate isomerase; IDH, isocitrate dehydrogenase; ACO, aconitase; G3P, glyceraldehyde-3-phosphate dehydrogenase; PE2, phenylalanyl-leucine peptidase; AK, adenylate kinase; MDH, malate dehydrogenase; PGD, gluconate-6-phosphate dehydrogenase; M1P, mannitol-1-phosphate dehydrogenase; GOT, aspartate aminotransferase; BGA, β-galactosidase; ADH, alcohol dehydrogenase; MPI, mannose-phosphate isomerase; G6P, glucose-6-phosphate dehydrogenase; IPO, indophenol oxidase; CAK, carbamate kinase; NSP, nucleoside phosphorylase; TDH, threonine dehydrogenase; SKD, shikimate dehydrogenase; GLU, glutamate dehydrogenase.

tries and over a period of more than 30 years. The majority of the O119 strains carried the virulence genes *eaeA*, *bfpA*, and EAF characteristic of typical EPEC, and none of them reacted with the probes for toxin genes. With the exception of one strain, all the isolates hybridized with the *eaeA* probe. Clonal analysis based on multilocus enzyme electrophoresis demonstrated that O119 strains carrying the *eaeA* gene are divided into two clonal groups with different combinations of virulence genes and flagellar antigens: cluster A, with typical EPEC characteristics, and cluster B, with atypical EPEC properties (Fig. 2).

Most cluster A strains are serotype H6 and harbor EAF plasmids with the *bfpA* gene and the EAF region. The occurrence of nonmotile strains (H⁻) and of *eaeA*⁺ and *eaeA*⁺ *bfpA*⁺ strains in cluster A does not contradict this conclusion, because these strains are probably recently derived variants of the H6/*eaeA*⁺ *bfpA*⁺ EAF strains. We hypothesize that these nonmotile strains are a consequence of the mutational loss of expression of flagella (6, 19, 36), and the virulence variants result from the loss of either the entire EAF plasmid (*eaeA*⁺ strains) or the EAF region (*eaeA*⁺ *bfpA*⁺ strains). The derivation of the H⁻ strains from the H6 strains is supported by the

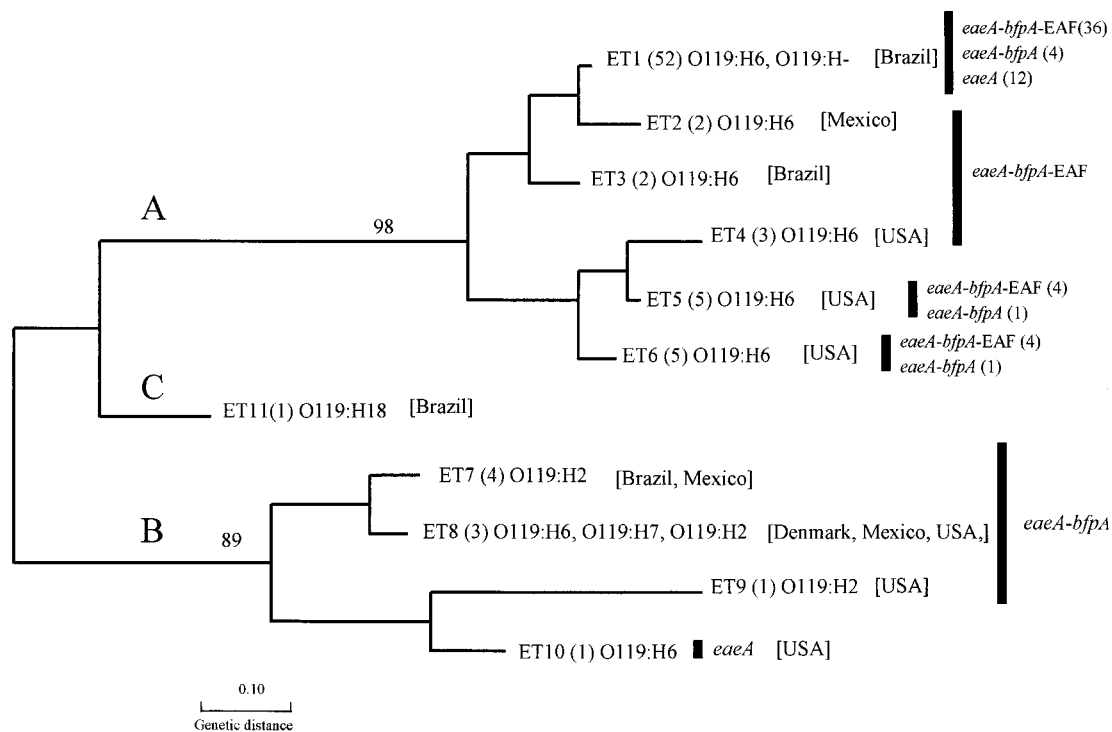


FIG. 2. Dendrogram of genetic relationship of 11 ETs of 79 representative strains of *E. coli* O119. Genetic distance is estimated in terms of electrophoretically detectable codon differences per enzyme locus. Serotypes, characteristic virulence determinants, and the origin of the strains (in brackets) are indicated on the right. The numbers of strains included in each ET and the number of strains displaying a particular gene combination are shown in parentheses. Numbers on the branches indicate the bootstrapping values supporting the corresponding genetic relationship represented.

occurrence of both types of strains in a single clone (ET1). The recent derivation of *eaeA*⁺ and *eaeA*⁺ *bfpA*⁺ strains from *eaeA*⁺ *bfpA*⁺ EAF⁺ strains is suggested not only because all combinations occur among isolates of the same ET (ET1, ET5, and ET6) but also because the H6/*eaeA*⁺ *bfpA*⁺ and the H6/*eaeA*⁺ strains were more frequently found among the O119 strains isolated before 1981, although the exact Fisher's test values were statistically significant only for the later group (Table 3). It is well established that genetic alterations often occur in *E. coli* strains stored in the laboratory for long periods (39). Thus, from these observations, we conclude that the common wild-type and presumably immediate ancestor of the cluster A strains has a motile H6 phenotype with the combination *eaeA*⁺ *bfpA*⁺ EAF⁺, which is characteristic of EPEC (17). Although the loss of virulence genes seems to be a plausible explanation, it cannot be ruled out that *eaeA*-*bfpA*⁺ EAF⁺ strains are recently derived from *eaeA*⁺ or the *eaeA*⁺ *bfpA*⁺ parental strains via the repeated acquisition of EAF plasmids (36).

In contrast to cluster A, cluster B strains typically have serotype O119:H2, with the exception of two O119:H6 strains and one O119:H7 strain. Except for a single strain with ET10, all strains of cluster B have an EAF plasmid lacking the EAF region. The absence of strains with a complete EAF plasmid (both EAF⁺ and *bfpA*⁺) in cluster B and the greater frequency of the H2/*eaeA*⁺ *bfpA*⁺ strains among those isolated more recently (after 1981) suggest that they belong to a naturally occurring stable clonal group. However, this difference in the frequency of H2/*eaeA*⁺ *bfpA*⁺ strains was not statistically significant (Fisher's test, $P = 0.160$). If this is so, we postulate the existence of two kinds of EAF plasmids in O119 strains, one with both the *bfpA* gene and the EAF region and the other with only the *bfpA* gene. The first kind would be typical of cluster A (O119:H6), and the second would be typical of cluster B (O119:H2). *bfpA*⁺ EAF⁻ plasmids are also observed in other EPEC serotypes such as O128:H2 and O142:H6 (unpublished data). The association of virulence plasmids with other *E. coli* serotypes seems to be very common (14, 43).

The third group of O119 cluster consists of a single strain which does not carry the *eaeA* gene. Interestingly, this strain is only distantly related by ET to the other *eaeA*⁺ strains. Because our sample includes 110 O119 strains isolated from many sources for 30 years, this kind of non-EPEC O119 strain can be considered a very rare natural *E. coli* population.

There was a close relationship between individual ETs and the geographical origin of the strains. However, although the three O119 clusters showed clear-cut differences in phenotypic and virulence properties, these traits were not associated with any geographical region, since distinct ETs from different clusters were found in single locations. This could suggest an adaptive irradiation of the two main divergent clonal groups.

According to the recent EPEC definition proposed by Kaper (17), cluster A strains with serotype O119:H6 and their non-motile derivatives meet the criteria of typical EPEC and cluster B strains with serotypes O119:H2 and O119:H7 do not fulfill these criteria since, although they have the EAF plasmid, they lack the EAF region.

Our observations indicate a close relationship between the combination of virulence genes and adherence phenotypes (Table 2). The LA pattern of the *eaeA*⁺ *bfpA*⁺ EAF⁺ strains may be explained by the presence of the EAF plasmid, and the LAL pattern of the *eaeA*⁺ strains may be explained by the absence of this plasmid. However, since the *eaeA*⁺ *bfpA*⁺ strains in this study carry the EAF plasmid (data not shown), the LAL pattern may be due to the lack of BfpA expression observed in these strains. As described here, 3 (2 H6 and 1 H⁻)

LA *eaeA*⁺ *bfpA*⁺ strains produced BfpA but none of 12 LAL *eaeA*⁺ *bfpA*⁺ strains produced the BfpA subunit. The eventual relationship between the absence of the EAF region and the failure to express BfpA is under investigation in our laboratory. In contrast to the correlation between the combination of virulence genes and adherence phenotype, most strains attach intimately as detected by the 3-h FAS test. This finding suggests that expression of BfpA may facilitate but is not required for intimate adherence to be detected in a 3-h FAS assay. It should be noted, however, that strains positive in a 6-h FAS assay occurred only among H6 strains of cluster A, what suggests that there are differences between the H6 serotype and the other serotypes. At present, we have no explanation for some discrepancies such as the observation of three *eaeA*⁺ *bfpA*⁺ EAF⁺ strains in which the adherence pattern could not be defined and two *eaeA*⁺ strains, one of which was nonadherent and the other displayed an indefinite adherence pattern.

The capacity of *E. coli* serotype O119:H6 to cause intestinal infection is supported by several epidemiological studies (13, 45) and by its genetic and phenotypic virulence characteristics. Furthermore, O119:H6 was one of the first EPEC serotypes found in association with attaching-and-effacing lesions in the intestinal mucosa of children with diarrhea (37). At present, it is not known if the H2 and other O119 serotypes with similar characteristics have the ability to cause diarrhea, since they bear an incomplete EAF plasmid and adhere slowly to HEp-2 cells and there are no epidemiological studies showing their association with diarrheal disease. However, the ability of serotype O119:H2 to cause intestinal infection cannot be ruled out, because it has been shown that an EPEC strain cured of the EAF plasmid, although less virulent than the wild-type strain, retained the capacity to cause diarrhea in volunteers (25). Furthermore, EPEC strains lacking the EAF plasmid are relatively frequent in individuals with diarrhea in whom no other pathogen can be identified (42). An EAF⁻ *eaeA*⁺ strain also has been implicated as the causative agent of an outbreak of diarrhea (46).

Further evidence for the role of O119 strains in infantile diarrhea comes from comparisons of the results presented here to our previous findings for the O55 and O111 serogroups. Virtually all of the O119 strains examined here are closely allied to one of two major clonal groups of EPEC strains, both of which have the *eaeA* gene and the EPEC virulence plasmid and cause AE lesions. The electrophoretic profiles of the cluster A O119 strains are most closely related to those of the classical EPEC strains of serotype O55:H6 (Table 2 of reference 36). Most of these LA⁺ O55 strains belong to a distinct cluster with the same virulence properties (*eaeA*⁺ EAF⁺ *bfpA*⁺) as the cluster A O119:H6 strains (Table 4; this study). Bacteria of other EPEC serotypes have also been shown to be part of this clonal group, referred to as EPEC 1 (47). In contrast, the O119:H2 strains of cluster B are most similar to O111:H2 (called ET 12 in reference 6). An O111:H2 strain is the most common EPEC strain recovered in Brazil. The H2 antigen also tends to be conserved among other EPEC serotypes of this cluster, which is referred to as EPEC 2 (47). However, some strains of this group have an EAF plasmid with the EAF region intact (i.e., O111:H2) whereas other members have a plasmid without this region (i.e., O119:H2). More work is needed on the diversity and structure of the EPEC virulence plasmids within these EPEC groups.

Finally, it should be emphasized that because of the clonal nature of EPEC, in serogroup O119 as well as in other EPEC serogroups (3, 6, 36, 50), genetic markers, virulence properties, and O and H antigens are highly correlated, which means that

in practice, serotyping may be useful for identification of EPEC clones.

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