INFECTION AND IMMUNITY, Sept. 1988, p. 2467–2473
0019-9567/88/092467-07$02.00/0

Vol. 56, No. 9

Genetic Relationships among Pathogenic *Escherichia coli* of Serogroup O157

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Received 19 January 1988/Accepted 7 June 1988

***Escherichia coli* strains of serotype O157:H7 are a newly described clonal pathogenic form associated with recent outbreaks of hemorrhagic colitis in humans. Although O157 strains of various H types have long been recognized as enterotoxigenic in animals, little is known about how these pathogenic animal strains are related to those of serotype O157:H7. To determine the genetic relatedness of O157:H7 isolates to animal O157 strains, we examined 194 O157 isolates, representing 12 distinct flagellar antigens (H serotypes), obtained from a variety of animal and human infections. To characterize isolates, we assayed allelic variation at 19 enzyme loci by multilocus enzyme electrophoresis. Genotypic comparisons of isolates revealed extensive variation among 33 distinct clonal genotypes that differed, on average, at 44% of the enzyme loci. K88 fimbriae were expressed in 72% of the isolates and occurred in a diversity of chromosomal genotypic backgrounds. Five major clonal groups were recognized; one group was clearly associated with porcine colibacillosis, and another was associated with human urinary tract infections. The O157:H7 genotype was not closely allied with any of the major groups of clones. The results indicate that O157 *E. coli* are genetically diverse and strongly suggest that the O157:H7 lineage was not recently derived from other pathogenic strains of the O157 serogroup.**

Strains of serotype O157:H7 have been recently recognized as a new pathogenic form of *Escherichia coli* through both epidemiological associations with outbreaks of hemorrhagic colitis and hemolytic-uremic syndrome in North America and Great Britain (10, 12, 13, 25, 27, 28, 33, 36, 41; W. M. Johnson, H. Lior, and G. S. Bezanson, Letter, Lancet i:76, 1983; S. Ratnam and S. B. March, Letter, J. Infect. Dis. 153:1176, 1986) and their ability to cause enteric disease in laboratory animals (2, 8, 26, 40; J. J. Farmer et al., Letter, Lancet i:702–703, 1983). Despite substantial progress in elucidating the mechanisms of virulence of O157:H7 strains (6), including the discovery of Shiga-like toxin genes (4, 16, 18, 38; A. D. O'Brien et al., Letter, Lancet i:702, 1983) and plasmid-encoded adherence factors (11, 15, 39), critical questions concerning the epidemiological and evolutionary origins of these putative pathogens are unanswered.

A population genetic analysis utilizing multilocus enzyme electrophoresis has revealed that O157:H7 isolates from diverse sources in North America belong to a bacterial clone that is unrelated to other Shiga-like toxin-producing clones of different serotypes (43). The combination of rare phenotypic traits for *E. coli* exhibited by O157:H7 strains may provide clues for tracing the evolutionary origin of these pathogens. One possibility is that O157:H7 strains are closely related to, and recently derived from, other pathogenic strains of the O157 serogroup.

E. coli O157 strains of various flagellar antigen types (H types) have long been recognized as enterotoxigenic in animals (17), especially as a cause of porcine colibacillosis (9, 24, 44). Many O157 animal isolates express K88 fimbriae (F4), adhesins known to be associated with the virulence of numerous serotypes that cause enteric disease in pigs, and elaborate enterotoxins, either heat labile or heat stable (44). O157 strains have also been recovered from human urinary tract infections (UTI) (3). A preliminary study of enzyme polymorphism revealed several distinct genotypes among a small number of animal strains of the O157 serogroup (20).

The objective of the present study was to assess the overall extent of genetic divergence among O157 strains obtained from animal and human infections and to establish the relationships of these strains to those of the O157:H7 clone. To address the hypothesis that O157:H7 strains were recently derived from a lineage of O157 strains, we used polymorphic enzymes, detected by multilocus protein electrophoresis (29), as genetic markers to characterize chromosomal genotypes of isolates and to determine the genetic relationships among diverse strains of serogroup O157.

MATERIALS AND METHODS

Bacterial isolates. A total of 194 *E. coli* strains were obtained from the *E. coli* Reference Center (ECRC) collection at Pennsylvania State University, which contains more than 20,000 serotyped *E. coli* cultures. We searched all records in the reference collection and compiled an exhaustive list of serotypes O157 cultures (n = 194). For these cultures, all available information on K and H serotypes, host species, tissue of isolation, geographical locality, and disease diagnosis was obtained. Strain ECRC 1075, which was originally isolated from hamburger meat implicated in a 1982 outbreak of hemorrhagic colitis in Michigan, was used to represent the O157:H7 clonal group identified in an earlier study (43). A derivative of K-12 (EC-1; Sigma Chemical Co., St. Louis, Mo.) was included for comparative purposes.

Antiserum and serotyping. The identification of O somatic antigens and H flagellar antigens was performed by agglutination methods adapted from standard procedures (7, 24). Isolates were cultured in veal infusion-yeast extract medium for 18 h and then heated at 100°C for 60 min for O serogrouping. Flagellar antigens were determined after the motility of the strains had been enhanced by growth in semisolid medium. All strains were evaluated against polyvalent pools and selected antisera with specific activity against 167 O and 54 H antigens.

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The K88 fimbrial antigen was identified by slide agglutination with a monoclonal antibody assay after the strains had been propagated in a special medium to enhance fimbrial expression (44).

Enzyme electrophoresis. To analyze enzyme electrophoretic variation, we subjected protein extracts from disrupted cells to horizontal starch gel electrophoresis and selective enzyme staining as described previously (29).

For each enzyme, electromorphs were distinguished by differences in the migration rates of specifically stained protein bands, and the bands were numbered in order of decreasing rate of anodal migration. The procedure was repeated for 19 enzyme systems (see Table 3) until the complete profile of electromorphs was resolved for every isolate. Isolates that lacked detectable enzyme activity were assigned a null allelic state at the locus in question.

Statistical analysis. Electromorphs of an enzyme were equated with alleles at the corresponding structural gene locus so that each bacterial strain was fully characterized by its multilocus genotype (allele combination) for the enzyme-encoding loci assayed. Genetic diversity was calculated for each enzyme locus as $h = n(1 - \Sigma p_i^2)/(n - 1)$, where p_i is the relative frequency of the i^{th} allele at a locus and n is the total number of isolates in the sample (29).

Distinctive multilocus genotypes were designated electrophoretic types (ETs) and were numbered by their inferred relationships from a cluster analysis (see below). Single-locus genetic diversity among ETs was calculated by the formula given above, with allele frequencies being tabulated over ETs and n being equal to the total number of ETs.

Cluster analysis based on the average-linkage algorithm was used to analyze genetic relationships among ETs (35, 43). A matrix of genetic distances between all pairs of ETs was calculated from the electrophoretic profiles. Each entry in the distance matrix was equal to the proportion of enzyme loci for which two profiles had different allelic states. For each pairwise comparison, loci with null states were not included, so that the resulting genetic distance yielded an estimate of the probability that two multilocus genotypes had different alleles at the average locus.

The statistical significance of groupings provided by the cluster analysis was assessed by a bootstrap analysis. For specific group sizes (n = 2 to 30), ETs were selected at random with replacement and the average genetic distance between genotypes was calculated. This procedure was repeated for 2,000 replicates for each group size, and the 5% critical value of distance was obtained. This value defined the distance below which 5% of the values fell in the distance distribution for randomly constructed groups of a given size. The observed distance for every cluster in the dendrogram was then compared with the critical values generated by the bootstrap procedure. Clusters with distances less than the the 5% critical value, given the size of the cluster, were considered statistically significant.

RESULTS

Characteristics of O157 isolates. For 186 of the 194 isolates there were complete records on the host species and disease diagnosis. Most O157 isolates (77%) were collected from cases of colibacillosis in pigs, and four additional colibacillosis-associated isolates were recovered from cows, a horse, and a goat (Table 1). Seven of the bovine isolates were associated with mastitis, pneumonia, or meningitis. Isolates were also recovered from human patients with UTI. A total of 21 isolates were obtained from feces from apparently healthy animals; for pigs, cows, and chickens, these strains were cultured from feces at the time of slaughter. A single isolate was obtained from a fecal sample from a healthy human. The remaining isolates included the representative O157:H7 isolate (ECRC 1075) and seven isolates with incomplete histories.

TABLE 1. Epidemiological sources of *E. coli* O157 isolates

Disease diagnosis	No. of isolates from indicated source:					
	Porcine	Bovine	Human	Avian	Equine	Caprine
Colibacillosis	142	2	0	0	1	1
Mastitis	0	5	0	0	0	0
UTI	0	0	9	0	0	0
Pneumonia	0	1	0	0	0	0
Meningitis	1	1	0	0	0	0
Abcess	0	0	0	0	0	1
None (fecal samples from healthy subjects)	10	5	1	6	0	0

A total of 186 isolates were serotyped for flagellar antigens (H types) and tested for the expression of K88 fimbriae (Table 2). Of these isolates, 99 were H43, and most of these (94 of 99) expressed K88 fimbriae ($K88^+$). Sixty-eight other strains were nontypeable or nonmotile and were $K88^+$ or $K88^-$ (did not express K88 fimbriae) in equal proportions. The remaining 19 isolates were of 11 different H types and were present in such low numbers that statistical analysis was irrelevant. However, in two cases in which more than one strain was available, namely, H20 and H38, both $K88^+$ and $K88^-$ variants were found. When the data for non-H43 types were pooled, there was a significant association of H43 with expression of K88 fimbriae and a high frequency of nonmotility in isolates that did not express K88 fimbriae ($\chi^2 = 57.8$, df = 3, $P < 0.05$). Thus, these data indicated that most O157:H7 strains were $K88^+$ and that the remaining O157 strains were either H nontypeable or scattered among 11 different H types.

Allelic and genotypic diversity. Among the 194 isolates examined, 18 of 19 (95%) of the enzyme loci assayed were polymorphic, with the number of alleles per locus ranging from 1 for carbamate kinase to 15 for β-galactosidase (Table 3). The average number of alleles per locus was 5.0. Single-

TABLE 2. Expression of flagellar antigen (H type) and K88 fimbrial antigen in O157 isolates

Flagellar antigen	No. of isolates that were:	
	$K88^+$	$K88^-$
H6	0	1
H7	0	1
H8	1	1
H11	1	0
H16	1	1
H20	1	2
H31	0	1
H34	0	1
H38	2	3
H42	1	0
H43[a]	94	5
H52	0	1
Nontypeable	13	14
Nonmotile	19	22

[a] Twenty-four percent showed a weak H8 cross-reaction.

TABLE 3. Genetic diversity for 19 enzymes in *E. coli* O157 isolates

No.	Enzyme	No. of alleles[a]	Genetic diversity for:	
			Isolates[b]	ETs[c]
1	Glucose-phosphate isomerase	6	0.452	0.667
2	Isocitrate dehydrogenase	5	0.183	0.547
3	Aconitase	9	0.449	0.725
4	Glyceraldehyde-phosphate dehydrogenase	4	0.041	0.174
5	Phenylalanyl-leucine peptidase	4	0.394	0.551
6	Adenylate kinase	4	0.041	0.229
7	Malate dehydrogenase	4	0.126	0.278
8	Gluconate-6-phosphate dehydrogenase	7	0.210	0.511
9	Mannitol-1-phosphate dehydrogenase	3	0.350	0.504
10	Aspartate aminotransferase	2	0.010	0.061
11	β-D-Galactosidase	15	0.513	0.945
12	Alcohol dehydrogenase	6	0.408	0.786
13	Mannose-6-phosphate isomerase	6	0.325	0.703
14	Glucose-phosphate dehydrogenase	2	0.041	0.170
15	Indophenol oxidase	3	0.021	0.119
16	Carbamate kinase	1	0.000	0.000
17	Nucleoside phosphorylase	2	0.020	0.119
18	Threonine dehydrogenase	4	0.126	0.403
19	Shikimate dehydrogenase	8	0.369	0.792

[a] Average, 5.0.
[b] Average, 0.215.
[c] Average, 0.436.

locus estimates of genetic diversity for isolates ranged from 0 for carbamate kinase to 0.513 for β-galactosidase, with an average diversity of 0.215. This level of allelic diversity means that two O157 isolates, selected at random, may be expected to have different alleles, on average, at 22% of the enzyme loci.

Comparisons of the allele combinations observed in isolates revealed 33 distinct ETs (Table 4). The degree of genetic divergence among pairs of ETs was large, as indicated by the single-locus genetic diversity values over ETs (Table 3). Ten loci had diversity values greater than 0.50, which means that most genotypes had different alleles at these loci. The average genetic diversity over ETs was 0.436, a value which represents about 80% of the total diversity in *E. coli* species observed as a whole (32, 42).

Attributes of widespread ETs. Six multilocus genotypes were represented by multiple isolates (Table 4) recovered from diverse localities. Comparisons among isolates with the same ET revealed that particular genotypes tended to be associated with specific diseases. The most abundant O157 genotype, ET-14, was represented by 140 isolates (72% of all isolates). Nearly all of these ET-14 isolates carried H43 antigen, expressed K88 fimbriae, and were associated with porcine colibacillosis. Isolates of ET-21 were also obtained from cases of porcine colibacillosis, but these seven isolates did not have H43 antigen, and only two of the five isolates

TABLE 4. Allele combinations and reference isolates for 33 ETs among 194 isolates of the O157 serogroup of *E. coli*

ET	Reference isolate[a]	K88	H type[b]	Allele at enzyme locus[c]:																		No. of isolates	State	Source	Disease association
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19				
1	820772	+	8	3	1	8	3	3	2	1	3	2	2	10	2	3	2	2	1	3	4	1	2	S.Dak.	Pig	Colibacillosis
2	850199	−	38	3	4	8	3	3	2	1	3	2	2	12	2	3	2	1	1	3	4	1	1	Pa.	Goat	Abcess
3	810370	−	8	3	4	8	3	3	2	1	3	2	2	13	0	3	2	2	1	3	4	2	1	Colo.	Pig	None (healthy)
4	861101	+	42	3	4	8	3	3	2	1	3	3	2	13	2	3	2	2	1	3	4	1	1	Nebr.	Pig	Colibacillosis
5	750560	+	43	3	4	7	3	3	2	1	3	2	2	11	2	1	2	2	1	3	4	1	1	Pa.	Pig	Colibacillosis
6	770021	−	NM	3	4	8	3	4	2	1	5	2	2	4	2	3	2	2	1	3	4	1	1	Pa.	Pig	Meningitis
7	790343	−	20	3	4	8	3	4	2	1	1	2	2	14	0	5	2	2	1	3	4	1	1	Colo.	Pig	None (healthy)
8	850351	−	NM	3	1	3	3	3	2	1	6	2	2	14	0	3	2	2	1	3	4	1	1	Va.	Pig	Colibacillosis
9	760038	−	NM	3	1	8	3	4	2	1	1	2	2	10	1	3	1	2	1	3	4	1	1	Pa.	Cow	Colibacillosis
10	790110	+	HN	3	1	8	3	4	2	1	3	2	2	12	2	3	1	2	1	3	4	1	1	Colo.	Cow	None (healthy)
11	851867	+	11	3	1	5	3	4	2	1	3	2	2	12	2	3	1	2	1	3	4	1	1	Nebr.	Pig	Colibacillosis
12	750473	+	NM	3	4	5	3	3	2	1	3	3	2	2	3	3	2	0	1	3	4	1	1	Pa.	Pig	Colibacillosis
13	800192	−	34	3	4	5	3	3	2	1	3	1	2	3	0	1	2	2	1	3	4	2	1	Colo.	Chicken	None (healthy)
14	750001	+	43	2	4	1	3	4	2	1	3	3	2	3	2	3	2	2	1	3	4	1	140	Pa.	Pig	Colibacillosis
15	851819	+	NM	2	4	1	2	4	2	1	3	3	2	3	0	3	2	2	1	3	4	2	1	Va.	Pig	Colibacillosis
16	750140	+	16	3	1	8	3	3	2	1	3	2	2	7	1	5	2	2	1	3	1	4	1	Pa.	Pig	Colibacillosis
17	790478	−	16	3	1	8	3	3	2	1	3	2	2	0	2	5	2	2	1	3	1	4	1	Colo.	Pig	None (healthy)
18	810593	+	43	3	4	5	3	2	2	1	3	2	2	12	1	5	2	2	1	3	1	3	3	Pa.	Pig	None (healthy)
19	790123	−	HN	4	4	5	3	2	2	1	3	2	2	12	0	5	2	2	1	3	1	0	1	Colo.	Cow	None (healthy)
20	750386	−	31	4	4	6	3	2	3	1	3	2	2	5	5	3	2	2	1	3	4	3	1	Pa.	Pig	Colibacillosis
21	750520	+	HN	4	4	5	3	3	2	1	3	3	2	8	3	5	2	2	1	3	4	4	7	Pa.	Pig	Colibacillosis
22	790541	+	HN	4	4	5	3	3	2	1	3	3	2	9	4	5	2	2	1	3	4	4	1	Colo.	Chicken	None (healthy)
23	790118	−	HN	4	1	5	3	3	1	1	3	3	2	10	0	6	2	2	1	3	4	4	1	Colo.	Cow	None (healthy)
24	840311	−	6	4	5	5	3	3	1	1	3	3	2	9	4	3	2	2	1	3	2	4	1	Pa.	Pig	Colibacillosis
25	790452	−	38	4	5	5	3	3	2	1	3	2	2	4	4	2	2	2	1	3	1	3	4	Colo.	Chicken	None (healthy)
26	820046	−	HN	4	3	5	3	3	2	1	2	1	2	9	0	3	2	2	1	3	4	5	3	Calif.	Cow	Mastitis
27	820479	−	HN	4	4	9	3	3	2	1	2	2	2	9	4	3	2	2	1	3	4	5	1	Pa.	Cow	Mastitis
28	821075	−	7	3	3	5	3	3	2	1	3	1	1	7	4	4	2	2	1	3	1	1	1	Wis.	Meat	Hemorrhagic colitis
29	750533	−	HN	4	4	8	3	3	2	3	3	2	2	5	4	4	2	2	1	3	4	6	2	Pa.	Cow	Colibacillosis
30	810253	−	NM	4	4	8	3	3	2	3	4	2	2	6	4	4	2	2	1	3	4	6	8	La.	Human	UTI
31	830542	−	HN	4	4	2	3	1	4	4	4	2	2	0	4	4	2	2	1	3	4	7	1	Mich.	Human	UTI
32	821063	−	HN	6	4	4	3	2	2	3	3	2	2	1	4	4	2	2	1	1	3	4	1	Pa.	Cow	Mastitis
33	830587	−	NM	1	4	5	1	3	2	2	7	3	2	4	2	4	2	2	1	2	4	2	1	Ohio	Horse	Diarrhea

[a] Each reference isolate was chosen to represent the predominant serotype.
[b] Abbreviations: NM, nonmotile; HN, nontypeable.
[c] Numbers designate allelic mobility classes, with 0 representing no detectable enzyme activity. See Table 3 for order of enzyme loci.

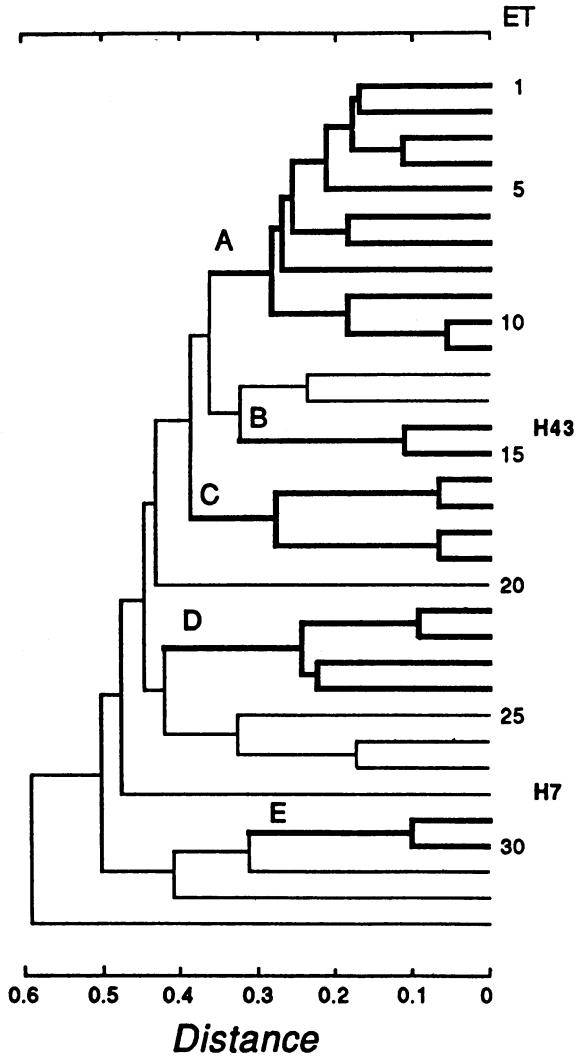

FIG. 1. Dendrogram portraying the average genetic distance between ETs. Bold lines designate significant groups determined by bootstrap analysis. The numerically dominant clone (ET-14) is designated by H43, and the O157:H7 genotype (ET-28) is designated by H7.

tested were K88$^+$. All 8 isolates of ET-30 were recovered from cases of human UTI, were nonmotile, and were K88$^-$. Isolates of ET-26 were associated with bovine mastitis, had H52 antigen (or were nonmotile), and were K88$^-$.

The two remaining O157 genotypes with multiple isolates were, for the most part, obtained from healthy animals and tended to be variable in H antigens. The genotype marked by ET-1 included two K88$^+$ strains, one which was associated with porcine colibacillosis and one of isolated from human feces. Strain K-12 also was classified as ET-1. All three isolates of ET-18 were obtained from apparently healthy animals and were K88$^+$. These strains included two bovine isolates with nontypeable flagellar antigens and a porcine isolate with H43 antigen.

Genetic relationships among ETs. To elucidate the genetic relatedness among the six widespread ETs and to estimate their relationships to O157 strains of other genotypes, we constructed a dendrogram derived from a cluster analysis of the genetic distance matrix (Fig. 1). A comparison of the observed clustering distances with values generated for randomly constructed groups revealed five major groups of ETs, labeled A to E (Fig. 1), that were significantly related at the 5% level. Within the five major groups, most subclusters had distance values less than the 5% critical value for each group size. Branches of the dendrogram not incorporated into one of these significant groups clustered at distances that are expected to occur more than 5% of the time by chance alone.

A key result of the classification of O157 strains into significant groups based on their ETs is that the ET marking the O157:H7 clone (ET-28) was not closely allied with any of the major O157 clusters. ET-28 entered a cluster at an average genetic distance of 0.47, which means that this genotype differed on average at more than 9 of the 19 polymorphic enzyme loci assayed (Fig. 1). At this distance, O157:H7 was not significantly related to any major branch within the O157 dendrogram.

Several noteworthy properties of the strains that constitute the four major groups are summarized in Table 5; strains not belonging to a significant group are listed under the heading "Others." There was significant heterogeneity among groups in the frequency of K88$^+$ isolates ($\chi^2 = 82.3$, df = 5, $P < 0.01$), nontypeable isolates ($\chi^2 = 28.2$, df = 5, $P < 0.01$), and nonmotile isolates ($\chi^2 = 21.0$, df = 5, $P < 0.01$). Isolates of group A (ETs 1 to 11) exhibited a variety of H types, and about one-half of the isolates expressed K88 fimbriae (Table 5). Isolates of four of the ETs in this group were obtained from healthy animals (Table 4), and all of these were closely related to K-12 (ET-1). Group B (ETs 14 and 15) included the numerically dominant ET of the O157 serogroup (ET-14). These isolates were virtually all recovered from porcine colibacillosis, were serotype H43, and represented the greatest percentage of K88$^+$ strains (89%) among the groups (Table 5). Group C (ETs 16 to 19) was represented by six isolates mostly isolated from healthy cows. Interestingly, one porcine isolate (ECRC 810253) of this genetically distinct group was O157:K88$^+$:H43 and thus was serotypically indistinguishable from ET-14 isolates. Isolates of group D (ETs 21 to 24) were mostly nontypeable for flagellar antigens, and three of the isolates were K88$^+$

TABLE 5. Characteristics of five major clonal groups of O157 strains

Clonal group	No. of ETs	No. of isolates	% K88$^+$	H type(s)	% H Nontypeable	% Nonmotile
A	11	13[a]	38	8, 11, 20, 38, 42, 43	23	23
B	2	141	89	43	8	19
C	4	6	33	16, 43	33	17
D	4	10	30	6	60	30
E	2	10	0		20	80
Others	10	15	33	7, 31, 34, 43, 52	33	13

[a] Includes the K-12 isolate (ET-1).

(Table 5). Isolates of ET-21, a widespread ET, were recovered primarily from porcine colibacillosis in geographically separate areas. Isolates of two of the other ETs of this group were from healthy animals (Table 4). Isolates of group E (ETs 29 and 30) were distinct because none expressed K88 fimbriae, and a high percentage were nonmotile (80%). This group included isolates associated with human UTI and two isolates from a bovine kidney infection. The remaining strains that were not classified into one of the five major groups represented a diverse collection of strains, 33% of which were H nontypeable and the others of which exhibited a variety of H types (Table 5).

DISCUSSION

Evolution of O157:H7 strains. Our previous analysis of O157:H7 isolates from a diversity of sources revealed that they constitute a genetically distinct clonal group that is only distantly related to Shiga toxin-producing strains of other *E. coli* serotypes (43). The key result of the present study is that *E. coli* O157:H7 isolates proved to be unrelated to any of 194 O157 strains isolated from animals and humans. The genetic distance between O157:H7 strains and the other major lineages exceeded 0.45, a level at which these strains cannot be assigned to a specific evolutionary branch within the O157 serogroup. This finding strongly suggests that the O157:H7 lineage was not recently derived from an ancestral O157 strain—clearly not from any we examined. Furthermore, the results suggest that if the O157:H7 clone has recently evolved from an extant *E. coli* lineage, then strains closely related to O157:H7 strains may be found in an O serogroup other than O157.

It has become clear that the putative virulence factors of O157:H7 strains, the ability to produce Shiga-like toxins and to adhere to epithelial cells (11, 39), also exist in other groups of *E. coli* (5, 14, 16). Because Shiga toxin genes and adherence factors may be transferred in the laboratory by bacteriophages or plasmids (11, 19, 34, 38, 39), these virulence factors potentially can spread horizontally in the natural *E. coli* population and thus come to occur in a wide variety of chromosomal backgrounds. Tzipori et al. (39) have recently demonstrated that neither the enteroadherence plasmid nor the capacity to produce Shiga-like toxins is essential for the virulence of O157:H7 in piglets, suggesting that other factors contribute to the virulence of O157:H7 strains. The discovery of *E. coli* strains that are closely related to O157:H7 strains may help clarify the role of the chromosomal genes in the pathogenic nature of O157:H7.

Clonal structure within the O157 serogroup. The genetic analysis of O157 isolates from diverse animal and human infections revealed five significant groups of ETs. Most O157 isolates collected throughout North America over a 10-year period belong to a single ET (ET-14), and these isolates usually express K88 fimbriae and carry the H43 flagellar antigen. Because these bacteria possess similar chromosomal genotypes, as indicated by their identical electrophoretic profiles, we hypothesize that they represent a naturally occurring clone and owe their similarity to descent from an ancestral cell. Isolates of the clone marked by ET-14 are generally associated with porcine colibacillosis and have not been recovered from healthy pigs or other animal species. In addition, this multilocus genotype occurs in a distinct lineage (group B; Fig. 1) that is highly divergent from that of other O157 genotypes.

The observation that several independent isolates from human UTI share a common ET suggests that these isolates also belong to a widespread clone, a finding that warrants further investigation into the virulence mechanisms of these isolates. The existence of an O157 clone causing UTI gained support through our recent finding that a strain from a child with pyelonephritis at Children's Hospital, Washington, D.C., which we predicted would fall into group E (Fig. 1) was identical in electrophoretic profile to ET-30. The clone marked by ET-30 was also significantly related to two clones isolated from bovine kidneys, suggesting that some common virulence factors may contribute to the pathogenic nature of these isolates. Furthermore, the genetic similarity of these isolates justifies further study of animal populations as reservoirs for human pathogenic *E. coli* strains.

The finding that K88 fimbriae occur in several distinct lineages and in so me cases are jointly expressed with H43 antigens on very diverse chromosomal backgrounds suggests that either $K88^+$:H43 is an ancestral condition or that strains have converged antigenically during their evolution. Because K88 fimbriae are encoded by plasmid-borne genes which can been transferred in the laboratory (23), horizontal gene transfer in nature is a plausible mechanism for phenotypic convergence.

Several evolutionary scenarios can be put forward to explain the occurrence of $K88^+$:H43 strains in diverse lineages. First, genes encoding K88 fimbriae and H43 flagellar antigens were cotransferred into diverse O157 strains. A simple mode for cotransfer would involve tight linkage between these genes on a single conjugative plasmid; however, there is presently no evidence that such plasmids occur in O157 strains. Second, K88 plasmids were transferred into O157 strains that did not have H43 flagella, and subsequent selection of flagellar characteristics in specific environments caused a conversion to H43 flagellar antigen. Third, K88 plasmids were transferred into strains that were neither O157 nor H43 serotype. The acquisition of K88 expression created new selection pressures for both the lipopolysaccharide and flagellar antigen properties, converting strains to the O157:H43 serotype. Further studies of K88 plasmids and the consequences of K88 transfers are necessary for distinguishing among these hypotheses.

The results presented here add to a growing body of evidence indicating that pathogenic *E. coli* strains have a clonal population structure (1, 20, 25, 30, 31, 37). The O157 antigen is shared by a diversity of strains subdivided into five major groups of clones; one group was clearly associated with porcine colibacillosis, and another was associated with human UTI. The O157:H7 genotype was not closely related to any clone of the serogroup, lending strong support to the hypothesis that O157:H7 strains were not recently derived from a lineage of the O157 serogroup.

ACKNOWLEDGMENTS

We thank Marie Wolfe for excellent technical assistance and Michael Davis of the ECRC for his role in the determination of serotypes. We are also grateful to R. K. Selander and two anonymous reviewers for comments on earlier versions of the manuscript.

This work was supported by Public Health Service grant AI24566 from the National Institutes of Health.

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