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Genetic relationships among *Escherichia coli* isolates causing urinary tract infections in humans and animals

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SUMMARY

Genetic variation in isolates of *Escherichia coli* obtained mostly from urinary tract infections in humans and domesticated animals (dogs and cats) was assessed for 16 enzymes using multilocus enzyme electrophoresis to characterize chromosomal genotypes. A total of 148 isolates comprised 63 distinct electrophoretic types (ETs) and about half of the isolates belonged to one of 9 common ETs. A bootstrap analysis of genetic distance between ETs revealed three significant groups of strains. Variation in allele frequencies among groups accounted for 40% of the total genetic diversity. The majority of the common ETs fell into a major cluster of closely related strains. The recovery of multiple isolates of the same electrophoretic types and serotypes from unassociated hosts suggests that these bacteria represent uropathogenic clones that are widely disseminated in humans and animals.

INTRODUCTION

Escherichia coli is the most frequently isolated organism from all types of urinary tract infections (UTI) and two hypotheses have been erected to account for its prevailing role in extraintestinal infection (Ørskov, 1981; Sussman, 1985). The prevalence hypothesis postulates that most UTI are opportunistic infections caused by bacteria that predominate in the faecal flora. This hypothesis grew from the observation that the same serotypes of *E. coli* commonly recovered in UTI are prevalent in the faeces at the onset of infection (Grüneberg & Bettleheim, 1969; Roberts *et al.* 1975).

The special pathogenicity hypothesis, in contrast, posits that most UTI are caused by specialized pathogenic strains that possess specific virulence factors which increase the likelihood of involvement in extraintestinal infections. This hypothesis has gained wide support from studies of the special pathogenicity features associated with uropathogenic *E. coli*. Typically uropathogenic strains exhibit a limited number of lipopolysaccharide (O) and acidic polysaccharide (K) antigens (Kaijser *et al.* 1977), express specific adhesins such as P-fimbriae (Källenius *et al.* 1981; Väisänen *et al.* 1984), and produce α- and β-haemolysins (Cooke & Ewins, 1975; Hughes *et al.* 1983). These factors are thought to play a role in attachment, ascent, and colonization of different tissue surfaces during progression of stages involved in infection of the urinary tract (Parry & Rooke, 1985).

Further support for the role of specialized pathogens in UTI comes from clonal analyses of virulent *E. coli* (see recent reviews by Achtman & Pluschke, 1986; Selander *et al.* 1987). For example, Caugant *et al.* (1983), who characterized UTI strains based on the electrophoretic variation of enzymes encoded by 13 chromosomal loci, found that the clonal composition of strains from symptomatic infections (i.e. cystitis and pyelonephritis) represented a highly non-random sample of the genetic population of the intestinal flora.

Although *E. coli* infections are a well-known cause of urogenital disease (cystitis, nephritis, metritis, prostatitis, and vaginitis) in dogs and cats (Morris & Sojka, 1985), few studies have focused on bacterial factors associated with pathogenicity of strains of animal origin (Westerlund *et al.* 1987; Garcia *et al.* 1988; Wilson *et al.* 1988). Garcia *et al.* (1988) demonstrated differences in the adhesins of F12 fimbriae produced by human uropathogenic strains and those produced by dog uropathogenic strains. Wilson *et al.* (1988) found a high prevalence of α – haemolysin and specific O antigens in urogenital strains from dogs and cats, and emphasized that similar characteristics are typical of strains associated with human UTI.

The purpose of the present study was to examine the genetic relationships among *E. coli* strains isolated from human UTI and animal urogenital infections (UGI). Our main objective was to estimate genotypic diversity among uropathogenic strains in humans and animals and to determine the extent to which isolates of similar genotype are recovered from infections in unassociated hosts. To address these issues, genotypes of bacterial isolates obtained from human UTI, animal (dogs and cats) UGI, and animal non-UGI extraintestinal infections were characterized by multilocus enzyme electrophoresis. The analysis of electrophoretic variation yielded a set of genetic markers for identifying clonal relationships among strains and for assessing the genetic relatedness of isolates from human and animal sources.

MATERIALS AND METHODS

Bacterial isolates. A total of 148 *E. coli* isolates, collected mostly from humans with urinary tract infections and domesticated dogs and cats with urogenital infections, was obtained from the *E. coli* Reference Center (ECRC) collection at Pennsylvania State University, University Park (Table 1). In addition to strains from dogs and cats with urogenital infections, the sample included 19 strains associated with other types of extraintestinal infections and 14 intestinal strains (Table 1). Strains from human UTI were isolated from patients in Michigan and Tennessee, whereas strains from animals were obtained from household pets in Florida. All isolates were originally collected between 1981 and 1985.

Enzyme electrophoresis. To analyse enzyme electrophoretic variation, extracts of water soluble proteins from disrupted cells were prepared and subjected to horizontal starch gel electrophoresis and selective enzyme staining as described previously (Selander *et al.* 1986).

For each enzyme, electromorphs (mobility variants) were distinguished by differences in the migration rate of specifically stained protein bands. The following 16 enzymes were analysed individually for mobility variants: glucose phosphate isomerase (PGI), isocitrate dehydrogenase (IDH), glyceraldehyde-

Table 1. *Epidemiological sources of 148* E. coli *isolates*

	Urogenital infection[1]	Other infections[2]	Intestinal strains	No. of isolates	No. of ETs
Human	91	0	0	91	37
Canine	16	9	11	36	21
Feline	8	10	3	21	13

[1] All human isolates from urinary tract infections (UTI), animal isolates from urogenital infections (UGI) include strains from UTI and vaginitis.

[2] Non-urogenital extraintestinal infections include isolates from lung, heart, spleen, conjunctiva, skin, and thoracic fluid in dogs and cats.

phosphate dehydrogenase (G3P), phenylalanyl-leucine peptidase (PE2), adenylate kinase (ADK), malate dehydrogenase (MDH), gluconate-6-phosphate dehydrogenase (6PG), mannitol-1-phosphate dehydrogenase (M1P), aspartate aminotransferase (GOT), alcohol dehydrogenase (ADH), mannose phosphate isomerase (MPI), glucose-6-phosphate dehydrogenase (G6P), indophenol oxidase (IPO), carbamate kinase (CAK), nucleoside phosphorylase (NSP), and threonine dehydrogenase (TDH). Electromorphs for each enzyme were numbered by rate of anodal migration. 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 loci, so that each bacterial strain was fully characterized by its multilocus genotype (allele combination) for the enzyme-encoding loci assayed (Selander *et al.* 1986). Distinctive multilocus genotypes were designated electrophoretic types (ETs) and were numbered by their inferred relationships from a cluster analysis based on the average linkage algorithm (Sneath & Sokal, 1973). For this analysis, a matrix of genetic distances between all pairs of ETs was calculated from comparisons of electrophoretic profiles. Each entry in the distance matrix was equal to the proportion of mismatches (i.e. the number of enzyme loci with different alleles divided by the number of loci compared) between two ETs. For each comparison, loci with null states were not included.

The statistical significance of clonal groupings furnished by the cluster analysis was assessed by a bootstrap analysis (Whittam & Wilson, 1988*b*). For specific group sizes ($n = 2, 3, \ldots, 30$), genotypes were selected at random with replacement from the list of 63 ETs and the average genetic distance between genotypes was calculated. This procedure was repeated for 2000 replicates for each group size and the 5% critical values of distance were obtained. These critical values defined the distance below which 5% of the values fell in the distribution for randomly constructed groups of different sizes. The observed distance for every cluster in the dendrogram was then compared to the critical values generated by the bootstrap procedure. Clusters with distances less than the 5% critical value, given the size of the cluster, were considered statistically significant.

Genetic differences between groups of ETs were assessed by partitioning the total genetic diversity into within- and between-group components (Selander *et al.* 1985). The genetic diversity for each locus within a group of ETs was calculated

as $h = n\,(1-\Sigma p_i^2)/(n-1)$ where p_i is the frequency of the ith allele in a group and n is the number of ETs in a group. The within-group diversity, H_s, was then calculated as the unweighted arithmetic average of the diversity values across groups. Total genetic diversity, H_T, was tabulated for each locus using the average allele frequencies across groups and the total number of ETs in the above formula. The coefficient of genetic differentiation among groups, G_{ST}, was calculated as $(H_T - H_S)/H_T$, which equals the ratio of the between-group component of diversity to the total genetic diversity across groups of ETs.

Antisera and serotyping. The identification of O somatic antigens and H flagellar antigens was performed by agglutination methods adapted from standard procedures (Edwards & Ewing, 1972; Ørskov *et al.* 1977). 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 strains were propagated in semisolid medium to enhance motility. All strains were evaluated against polyvalent pools and select antisera with specific activity against 167 O and 54 H antigens.

RESULTS

Enzyme polymorphism and multilocus genotypes. Among the 148 isolates, all 16 enzymes assayed exhibited polymorphism in electrophoretic mobilities. The number of electromorphs per enzyme (alleles per locus) averaged 4·8 and ranged from 2 for 4 enzyme loci to 11 for locus encoding gluconate-6-phosphate dehydrogenase (Table 2). A total of 63 distinct electrophoretic types (ETs) or multilocus genotypes was identified.

The total genetic diversity, H_T, for 63 ETs classified into four subdivisions based on source of isolation (human UTI, animal UGI, animal non-UGI, and animal intestinal strains) ranged from 0·725 for the most diverse locus encoding mannitol-1-phosphate dehydrogenase to 0·050 for the least variable locus encoding carbamate kinase. Genetic diversity among strains was lower for urinary infections (human UTI, $H = 0{\cdot}338$; animal UGI, $H = 0{\cdot}365$) than for non-urogenital infection ($H = 0{\cdot}385$) or intestinal strains ($H = 0{\cdot}460$). The total diversity averaged across loci equals 0·391 which means that two ETs selected at random from the pooled sample differ, on average, at about 40% of the enzyme loci.

There was no evidence for substantial genetic differentiation among isolates from human and animal sources, as reflected by the small values of G_{ST} in Table 2. The average G_{ST} among loci for ETs from different sources equals 0·010 which means that only 1% of the total single-locus diversity is accounted for by genetic differences between strains from human and animal sources.

Clonal groupings. The genetic relationships among 63 clones are summarized in a dendrogram generated by an average linkage cluster analysis (Fig. 1). The dendrogram reveals extensive divergence among groups of clones, with some ETs (i.e. ETs 61–63) differing at over 65% of their loci from the majority of strains in the sample.

From the results of the bootstrap analysis, which generated distributions of genetic distances for random groups of ETs, three major groups or lineages of closely related ETs were found to be significant at the 5% level (ETs 1–28, ETs 31–46, and ETs 49–55). Strains isolated from human and animal extraintestinal

Table 2. *Genetic diversity and coefficient of differentiation for 16 enzymes in* E. coli *across sources and groups.*

Enzyme[3]	No. of alleles	Source[1] ($n = 63$)		Group[2] ($n = 51$)	
		H_T	G_{ST}	H_T	G_{ST}
PGI	7	0·601	0·000	0·705	0·432
IDH	7	0·437	0·016	0·386	0·277
G3P	2	0·134	0·048	0·047	0·033
PE2	7	0·684	0·000	0·597	0·482
ADK	3	0·511	0·001	0·376	0·467
MDH	5	0·293	0·000	0·181	0·072
PGD	11	0·596	0·010	0·613	0·020
M1P	8	0·725	0·081	0·717	0·561
GOT	4	0·389	0·000	0·178	0·172
ADH	4	0·616	0·027	0·584	0·558
MPI	7	0·543	0·000	0·466	0·099
G6P	3	0·160	0·000	0·047	0·033
IPO	2	0·014	0·009	0·000	—
CAK	2	0·050	0·000	0·000	—
NSP	2	0·126	0·033	0·000	—
TDH	3	0·373	0·041	0·469	0·949
Average	4·8	0·391	0·010	0·335	0·401

[1] Subdivided into four sources – human UTI, animal UGI, animal non-urogenital extra-intestinal infections, and animal intestinal strains.

[2] Subdivided into three groups denoted by bold-lined clusters in Fig. 1.

[3] See text for identification.

infections – denoted by solid circles for human UTI (H), animal UGI (U), or animal other infection (O) – occur throughout the dendrogram illustrating the previous finding of insignificant genetic differences between strains from different sources. In contrast, ETs in the three major groups of the dendrogram exhibit substantial genetic divergences as demonstrated by the relatively large value of G_{ST} (Table 2). For the 51 ETs that fall into the three major groups, about 40% of the total diversity is accounted for by genetic variation among these distinct lineages.

Three of the ETs in the dendrogram (ET-1, ET-14, and ET-21) were frequently isolated and were recovered from extraintestinal infections in both humans and animals. Of the 22 ETs with multiple isolates, about 16% were recovered from both human UTI and animal UGI.

Serotypic characteristics of common ETs

Almost half of the isolates analysed in this study belonged to one of nine common ETs (Table 3). Eight of the nine common ETs fell into the major cluster (ETs 1–28) at the top of Fig. 1. The remaining common ET (ET-35) fell into the second major cluster (ETs 31–46). Three of the common ETs (ET-4, ET-6, and ET-9) were recovered only from human UTI, five were recovered from both human and animal infections, and one ET (ET-23) was collected from animal sources.

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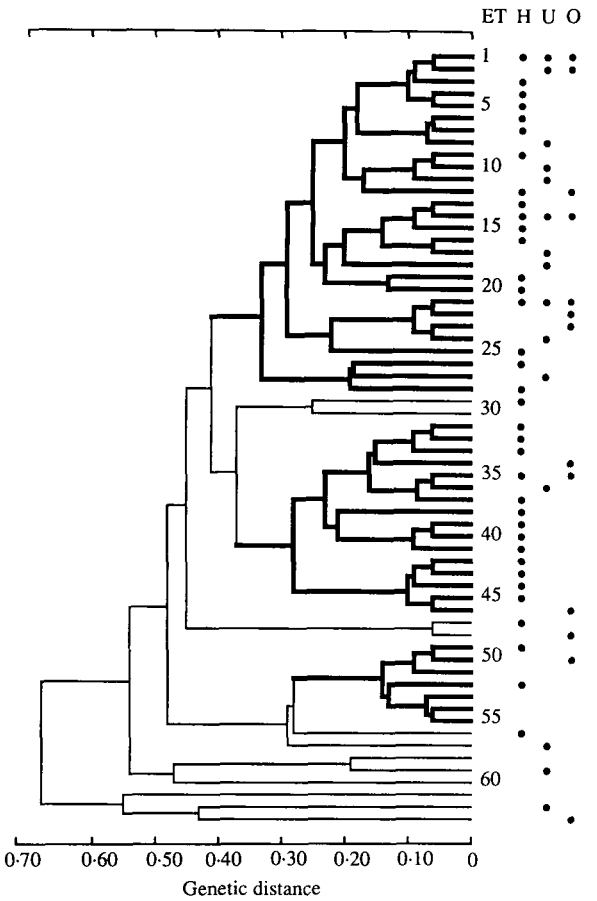

Fig. 1. Dendrogram of the genetic relationships among 63 *E. coli* clones. Three significant groups of ETs, determined by bootstrap analysis, are highlighted by bold lines (ETs 1–28, ETs 31–46, and ETs 49–55). The solid circles denote ETs recovered from human UTI (H), animal urogenital infections (U), and animal non-urogenital infections (O).

The data in Table 3 show a high level of concordance between electrophoretic type and serotype, as reflected by the following two observations. First, isolates of the same ET tend to have the same O:H serotype with only occasional rare antigenic variants observed. For instance, all of the isolates of ET-21 from both human and canine sources were serotype O6:H31 (Table 3) and most of the isolates of ET-1 were serotype O6:H1. A single isolate of ET-1, recovered from feline UTI, exhibited an O2 antigen rather than the O6 antigen expressed by other ET-1 isolates. The genotype marked by ET-14 was recovered from human, canine and feline infections and generally was serotype O4:H5; however, two of the human UTI isolates exhibited distinct O antigens (O13, O18). Second, all of the common ETs differed in the predominant O:H serotype; there was no case of the same O:H serotype (except for non-motile strains) being expressed by isolates of different common ETs.

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Table 3. *Serotypic variation and host distribution for nine common ETs*

ET	No. of isolates	Hosts	Predominant serotype[1]	Other serotypes
1	13	Human, dog, cat	O6:H1	O6:H2, O2:H−
4	5	Human	O18:H1	—
6	7	Human	O−:H−	O18:H−
9	4	Human	O2:H6	—
12	4	Human, dog[2], cat[2]	O4:H−	O4:H5
14	15	Human, dog, cat	O4:H5	O13:NM, O18:NM
21	16	Human, dog	O6:H31	—
23	4	Dog[3], cat[2]	O−:H31	O6:NM
35	4	Human, dog	X14:H45	O11:H15

[1] Small number of strains were non-motile or not definitively distinct from the numerically dominant O:H serotype. O− and H−, strains non-typeable with standard antisera; NM, non-motile.

[2] Non-UGI extraintestinal infection.

[3] Intestinal strain.

DISCUSSION

The genetic variation detected at enzyme-encoding loci through the application of multilocus enzyme electrophoresis has provided useful systems of genetic markers for analysing the population structure of pathogenic *E. coli* (Caugant *et al.* 1983; Ochman & Selander, 1984; Selander *et al.* 1985; Whittam *et al.* 1988; Whittam & Wilson, 1988*a*, *b*) and other bacterial species (reviewed by Selander *et al.* 1987; Selander & Musser, 1988). An important generalization emerging from these studies is that many bacterial pathogens have a clonal population structure with only a limited number of pathogenic clones accounting for most cases of serious disease (Selander & Musser, 1988). Our analysis of electrophoretic variation in *E. coli* strains from urogenital disease indicates that many isolates associated with infections in human and domesticated dogs and cats from separate geographic areas belong to a limited number of electrophoretic types, a result suggesting that these ETs mark multilocus genotypes that represent widespread uropathogenic clones.

Although there is extensive genetic diversity across all isolates examined in this study, only a small component (1%) of total diversity is accounted for by genetic differences in isolates collected from human, dogs, or cats. Thus, the clonal compositions of strains associated with human UTI, animal UGI, and animal non-UGI extraintestinal infections are apparently very similar. This finding is consistent with the observation that most canine and feline uropathogenic strains belong to the same limited number of O serogroups commonly found in human uropathogenic strains (Westerlund *et al.* 1987; Garcia *et al.* 1988; Wilson *et al.* 1988).

Evidence for uropathogenic clones. Early indications of the clonal nature of uropathogenic strains were based primarily on the observations that groups of strains associated with human UTI tended to share sets of attributes, especially specific O:K:H antigens (Mabek *et al.* 1971; Belsheim *et al.* 1981; Ørskov *et al.* 1982; Achtman *et al.* 1983). More recently, Väisänen *et al.* (1984) described seven clones associated with pyelonephritis in which independent isolates of each clone

tended to be homogeneous with regard to complete O:K:H serotype, fimbriation, haemolysin production, outer membrane proteins, and plasmid profile. Moreover, not a single representative of these clones was found among faecal strains from healthy children. The most convincing evidence indicating that much pyelonephritis is caused by a limited number of pathogenic clones was the finding that electrophoretic types associated with pyelonephritis from Finland and Sweden were, on average, more closely related to one another than to clones from the same geographic locality but from different clinical sources (Selander *et al.* 1987).

Interestingly, two of the common ETs (ET-1, ET-14) that were found in humans, dogs, and cats (Table 3), have the same O:H serotypes as two of the uropathogenic clones described by Väisänen *et al.* (1984) in their study of human UTI strains from Finland. Clone IV of Väisänen *et al.* (1984, table 5), associated with human pyelonephritis, has serotype O4:K12:H5 and expressed P fimbriae, Type 1 fimbriae, and haemolysin. We suspect that isolates of the clone marked by ET-14 (Table 3), which are predominantly serotype O4:H5, belong to the uropathogenic Clone IV lineage. In addition, isolates of the clone marked by ET-1 (Table 3) generally have the same O:H antigens as Clone V (O6:K2:H1) and Clone VIII (O6:K13:H1) described by Väisänen *et al.* (1984, table 5). Although further comparisons between these groups of strains are needed, the results at this point strongly suggest that certain uropathogenic clones, distinguished both by ET and O:K:H serotype, have achieved intercontinental distributions and have the ability to cause urogenital disease in humans, dogs, and cats.

Our results support and extend the earlier studies suggesting the existence of virulent clones with special ability to cause human UTI and other extraintestinal infections (Achtman *et al.* 1986; Selander *et al.* 1985; Achtman & Pluschke, 1986). The finding that independent isolates, collected from humans and animals in separate geographic regions, share similar genotypes, as identified by electrophoretic type, suggests that many cases of serious urogenital disease may be caused by a small number of uropathogenic clones. The clonal relationships described provide a framework for the further analysis of geographic distribution, host specificity, and virulence properties of uropathogenic clones.

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