Distribution of multilocus genotypes of *Escherichia coli* within and between host families

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SUMMARY

Isolates from the intestinal *Escherichia coli* flora of 28 members of five families (including parents, children, and household pets) in Amherst, Massachusetts, and Rochester, New York, were characterized by the electrophoretic mobilities of 12 enzymes to estimate the extent of sharing of strains among associated and unassociated hosts. Among the 655 isolates examined, 60 different combinations of electromorphs (electrophoretic types or ETs), each representing a distinctive multilocus genotype, were identified, of which 85 % were recovered from only a single individual. On average, 11 % of the ETs isolated from the same family were shared by two or more members; 4·9 % of ETs were shared among members of unassociated families living in the same city; and only 2 % were shared by families in different cities. All three ETs that were recovered from multiple hosts in the present study are widespread clones that have been isolated from many other host individuals in North America and Sweden.

INTRODUCTION

In a study of the genetic structure of the intestinal *Escherichia coli* flora of an individual human host over an 11-month period, Caugant, Levin & Selander (1981) identified 53 distinctive multilocus genotypes by electrophoresis of 15 enzymes. This finding supported and extended earlier evidence, from serotyping, that individual hosts may harbour large numbers of strains (Cooke, Ewins & Shooter, 1969; Bettelheim *et al.* 1977; Shooter *et al.* 1977). With the exception of two strains that were resident in the individual host throughout most of the sampling period, there was a rapid turnover of strains, apparently as a consequence of continued immigration and extinction of new types from external sources. Little if any of the genotypic diversity of the host's flora could be accounted for by *in situ* genetic recombination among either the resident or transient strains.

Apart from cases of local epidemic diarrhoea in which the same strain has been recovered from many patients (for example, the occurrence of the strain typed as 0148 in British soldiers travelling in Aden (Rowe, Taylor & Bettelheim, 1970)),

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there is a little information on the extent of sharing of strains of *E. coli* among either associated or unassociated hosts (Sears & Brownlee, 1952; Cooke, Ewins & Shooter, 1969; Bettelheim *et al.* 1974 *a, b*; Selander & Levin, 1980). However, the repeated recovery of certain serotypes and bio-serotypes from widely separated host populations has led to the hypothesis that at least some strains, and particularly those associated with human septicaemia, meningitis and neonatal diarrhoea, represent clones (asexual cell lines) of worldwide distribution (Ørskov *et al.* 1976, 1977; Bettelheim, 1978). For certain K1 isolates of several O serogroups from Europe and the United States, this hypothesis has recently been confirmed by studies of outer membrane proteins and biotypes (Achtman *et al.* 1983), the electrophoretic mobilities of enzymes (Ochman & Selander, 1984), the structure of the O group lipopolysaccharides and other antigenic characters (Kusecek *et al.* 1984), and the distribution of plasmids (Silver *et al.* 1980). Whether the clone hypothesis of genetic structure can be extended to populations of *E. coli* in general remains to be determined by further studies of the distribution of strains among human and other hosts.

We here report the results of a study of the extent of sharing of strains, identified by multilocus enzyme electrophoresis, among members of five human families (including household pets) in two geographic areas of the northeastern United States.

MATERIALS AND METHODS

Isolation and identification of E. coli. We obtained 34 faecal samples on sterile cotton swabs from 28 individual hosts belonging to three families in Amherst, Massachusetts, and two families in Rochester, New York. Families I and II lived in the same neighbourhood, and their members were frequently associated on social occasions; but there was no association of members of the other families studied. The host individuals sampled included two parents, one or two children, and one or two pet dogs and cats. All individuals in a family were sampled in a period of from one to four days; and family I, in Amherst, was sampled twice at an interval of three months (May and August, 1979).

The health records of host individuals were not determined in detail, but, except for the daughter of family III, who had been taking tetracycline for several years, and the cat of family I, which was treated with antibiotics one month before the first sample was taken, none of the subjects yielding *E. coli* is known to have received antibiotic treatment for a period of at least several months prior to the time our samples were taken.

Faecal samples were suspended in buffer and streaked on lactose minimal (ML) plates, from which 10–33 single-colony isolates were taken at random, for a total of 655 isolates. Each isolate was streaked for two cycles on complete medium (tetrazolium lactose) and tested for growth on citrate-minimal medium plates. Citrate-negative colonies were stored in stabs for electrophoresis. In the case of family III, where we expected to find tetracycline-resistant strains, we isolated strains on ML–tetracycline plates (25 μg/ml tetracycline) as well as on ML plates.

A faecal sample taken from a third dog in family V yielded no isolate of *E. coli* (similar cases were mentioned by Cooke, 1974); and the predominant coliform

bacterium was *Klebsiella pneumoniae* (identified by the API 20 E test). This dog had been treated with antibiotics only a few days before the faecal sample was taken.

Electrophoretic procedures. Methods of preparation of aqueous protein extracts and of starch-gel electrophoresis of enzymes were described by Caugant, Levin & Selander (1981). Twelve enzymes were assayed for electrophoretic mobility: malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PD), 6-phospho-gluconate dehydrogenase (6-PGD), alcohol dehydrogenase (ADH), isocitrate de-hydrogenase (IDH), phenylalanyl-leucine peptidase (PE2), leucyl-glycyl-glycine peptidase (PE3), phosphoglucose isomerase (PGI), mannose-6-phosphate isomerase (MPI), aconitase (ACO), adenylate kinase (AK), and β-galactosidase (βGA). Modifications of the buffer system were made for MPI and AK, for which Poulik and Tris-citrate, pH 8·0, were used, respectively.

Each unique combination of electromorphs (allozymes) of the 12 enzymes was designated as an electrophoretic type (ET) (see Table 1 and Caugant, Levin & Selander, 1981, for details). ETs were sequentially numbered in chronological order of their recovery and identification from hosts. For purposes of calculating genic diversity, electromorphs of an enzyme were equated with alleles at the corresponding structural gene locus.

RESULTS AND DISCUSSION

Genic and genotypic diversity. Among the 655 isolates assayed for electrophoretic variation at 12 enzyme loci, 60 different electrophoretic types (ETs) were identified. The mean genic diversity per enzyme-encoding locus among the 60 ETs (calculated as the mean over loci of $1 - \sum\limits_{i=1}^{e} x_i$, where x_i is the frequency of the ith electromorph of an enzyme) was 0·50. (In this calculation, an ET was counted only once for each host from which it was isolated but as many times as it occurred in different hosts.) This value is closely similar to those obtained in two earlier surveys of genic variation in *E. coli* from a wide variety of hosts, based on analyses of 20 and 12 enzyme loci, respectively (Selander & Levin, 1980; Ochman *et al.* 1983). However, it is larger than the estimate (0·39) obtained for ETs isolated from a single human host over an 11-month period (Caugant, Levin & Selander, 1981).

The number of isolates obtained from each host and the ET assignments of all isolates are shown in Table 1. Fourteen of the 34 faecal samples yielded only a single ET; and the number of ETs per individual host ranged from one to 11, with a mean of 2·3. Fifty-one of 60 (85 %) of the ETs were each recovered from only one host individual. Thirty-eight ETs were recovered exclusively from human hosts, 18 were isolated only from animals, and four were found in both humans and animals.

The relatively extensive data available for family I suggest that sharing of ETs among family members is not infrequent. Of the 25 ETs identified among the 227 isolates obtained from members of family I, seven (28 %) were recovered from two or more members. Thus ET-2 was isolated from the father, mother, son and daughter; ET-5 from father and mother; ET-10 from father, mother and dog; ET-16 from son and cat; and ET-14, ET-18, and ET-19 from dog and cat.

Only one (ET-33) of the 10 (10 %) ETs that were recovered from family III was

Table 1. *Distribution of 655 isolates of 60 electrophoretic types (ETs) among members of five families*

F. father; M. mother; D. daughter

| | Amherst, Mass. | | | | | | | | | | | | | | | | | | | | | | | | Rochester, N.Y. | | | | | | | | | |
| | Family I (May 1979) | | | | | | Family I (August 1979) | | | | | | Family II (June 1979) | | | | | | Family III (August 1980) | | | | | | Family IV (Jan. 1981) | | | | Family IV (Sept. 1980) | | | | | |
ET	F.	M.	Son	D.	Cat	Dog	F.	M.	Son	D.	Cat	Dog	F.	M.	D. 1	D. 2	Cat	Dog	F.	M.	Son	D.	Cat	Dog	F.	M.	D.	Cat	F.	M.	D. 1	D. 2	Dog 1	Dog 2
1	4																																	
2			11					18		20				17									6		16		20							
3	2																																	
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16			9						12		20						10																	
17				19																														
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31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	No. of isolates examined	No. of ETs
																		10	7	1	1									19	4
																			19											19	1
																	20													20	1
																20														20	1
																						3	1	1	2	1	9	3		20	7
														18	2															20	2
													20																	20	1
																														20	1
											1	15	4																	20	3
													4																	20	2
	14					1																								15	2
											23	2																		31	3
		33																												33	1
			8	9	9																									26	3
	15																													15	1
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																														19	2
																														21	11

Table 2. *Extent of sharing of ETs within and between families*[1]

	Percentage of shared ETs	
Level	Mean	Range
Within families	11·0	0–28
Between families		
Same city		
Associated	9·7	—
Unassociated	4·9	0–12
Different cities	2·0	—

[1] Based on total numbers of ETs identified within a group or groups at each level. E.g., within families, on the average, 11·0 % of ETs identified among isolates collected from all family members were shared by two or more members.

shared by two members; and for family IV, only one (ET-2) of six ETs (17 %) was shared by two members; none of the nine ETs from family II or the 16 ETs from family V was shared among members. For all five families, the mean proportion of ETs shared by two or more members was 11 % (Table 2).

For families I and II, which lived in the same neighbourhood and whose members were associated, a total of 31 ETs was identified, of which three (ET-2, ET-12, and ET-16), or 9·7 %, were recovered from individuals of both families. ET-2 was isolated from the father, mother, son and daughter of family I, as well as from the mother of family II; ET-12 from the father of family I and the father of family II; and ET-16 from the son and cat of family I and the dog of family II.

Note that only two of the 60 ETs were recovered from members of unassociated families. These are ET-2, which occurred in four of the five families, and ET-12, which was present in the associated families 1 and II and also in family III, all in Amherst.

Total genic diversity was apportioned within and between families according to the method of Jaenike & Selander (1980), which is a variant of a method introduced by Lewontin (1972). In view of the fact that most ETs were isolated from only a single host, it is not surprising that most (92·6 %) of the total genic diversity is attributable to variation within families. Diversity between families in each city accounted for only 6·2 % of the total, and the remaining diversity, 1·2 %, was attributable to varation between cities

In order to study the distribution of ETs on a larger geographic scale, we cross-classified each ET detected in the present study with those characterized in other surveys (Selander & Levin, 1980; Caugant, Levin & Selander, 1981; Caugant *et al.* 1983). About 12 % of the 60 ETs had been recovered in earlier studies, and most of these had been isolated from only one other host. It is noteworthy that the three ETs recovered in the present study from unassociated families have been found in the flora of many other hosts. ET-2, which was isolated from eight of the 28 hosts in Amherst and Rochester, is electrophoretically indistinguishable from the laboratory strain K-12, and has also been isolated from individuals in Iowa and Sweden. ET-16, which was recovered from both humans and animals in Amherst, was earlier detected in the faecal flora of two healthy girls and in the urine of 12 bacteriuric girls in Sweden. ET-12, which was obtained from the three

adult males in Amherst, was recovered in samples from 13 unrelated hosts in Sweden; and, in an earlier study, it was identified as a resident strain in the father of family I, where it persisted at least until March 1980 (Caugant, Levin & Selander, 1981). Because we suspected that ET-12 was also a resident in the fathers of families II and III, additional faecal samples were obtained from these individuals in March 1981, 9 and 7 months, respectively, after the initial samples were taken. And in each case isolates of ET-12 were recovered.

It is probable that our survey detected only those strains that were abundant in a hosts's flora at the time the faecal sample was taken. Moreover, the numerous genetically distinct strains that have thus far been identified by multilocus enzyme electrophoresis and by other techniques (e.g. serotyping, biotyping, phage resistance, bacteriocin assay) apparently represent only a small fraction of the total genotypic diversity in the species *E. coli* as a whole (Ochman *et al.* 1983). Evidence from the present study supports the growing conclusion that some genotypes are geographically very widespread (perhaps worldwide) in distribution, whereas others have more restricted ranges. These findings are in accord with the clone concept, originally proposed by Ørskov *et al.* (1976, 1977) on the basis of the widespread occurrence in pathogenic strains of a limited number of serotypes and biotypes. More recent work has shown that K1 strains of several O serogroups are widely distributed in both Europe and North America and have remained temporally stable over periods of at least 40 years (Achtman *et al.* 1984; Ochman & Selander, 1984). We presently do not know the extent to which these more successful clonal types have special characters, e.g. adherence to intestinal epithelial cells, that promote their widespread distribution and persistence.

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