Epidemiol. Infect. (1991), **107**, 537–542
Printed in Great Britain

The population structure and transmission of *Escherichia coli* in an isolated human community; studies on an Antarctic base

Y. TZABAR* AND T. H. PENNINGTON

Department of Medical Microbiology, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD

(*Accepted 13 June 1991*)

SUMMARY

The population structure and transmission of *Escherichia coli* in a small group of individuals isolated for 26 weeks on an Antarctic base were studied by multilocus electrophoresis of eight enzymes and plasmid analysis. Two hundred and sixty-nine strains were isolated. They were grouped into 60 allozyme types (ETs). Half of these ETs were only isolated once; others were repeatedly isolated from single subjects. Eleven were found in more than one subject and the pattern of the occurrence of some of them was considered to provide evidence of their spread from subject to subject.

INTRODUCTION

Recent work on *Escherichia coli* has shown that populations of this organism in man and animals show extensive genetic diversity, with the underlying genetic structure being that of an array of stable cell lineages (clones), among which there is little recombination of chromosomal genes [1]. Much of the evidence supporting these conclusions comes from work using the classical population-genetic technique of multilocus enzyme electrophoresis, which has been used to study the change in the *E. coli* population in an individual over time [2], the differences in *E. coli* populations between individuals over geographical distance [3] and the transmission of pathogenic strains [4]. Multilocus electrophoresis has the advantage over serotyping in that genotypic data can be obtained for all isolates. Additionally, because most of the enzyme loci so far examined in *E. coli* are represented in populations by many alleles, the power of the technique to resolve genetic differences between strains is very high [5].

The Antarctic bases of the British Antarctic Survey have features which make them particularly suitable for studies on the population structure of bacterial commensals of man and the transmissions of these organisms from host to host. Their human populations are small and amenable to studies of this type, they live in a well-defined environment and, most importantly, they are isolated from external sources of bacteria for long uninterrupted periods.

This paper reports the results of a study which used multilocus electrophoresis and plasmid analysis to assess the diversity of *E. coli* and its transmission among

* Present address: Department of Anaesthetics, Aberdeen Royal Infirmary, Aberdeen.
† Address communications and reprint requests to Professor T. H. Pennington.

members of the British Antarctic Survey base on Signy Island (South Orkney Islands).

MATERIALS AND METHODS

Bacteria

Sixteen young adult healthy male subjects were studied during the Antarctic winter, when the base was completely isolated from the outside world for 26 weeks. Additional samples were taken before and after this period; a supply boat with about 30 individuals (who were not studied) was present at the base during the first month of the study. It arrived again about 1 week before the last samples were taken.

Faecal samples were collected using moistened swabs; each subject was sampled every 2 weeks during the 32-week study period. Presumptive colonies of *E. coli* on MacConkey agar were inoculated into thioglycollate transport medium and stored at 4°. After transport to Aberdeen isolates were subcultured and identified using the API-20 kit. A total of 1447 presumptive *E. coli* isolates were transported to the UK; 592 gave growth on subculture, of which 269 were identified as *E. coli*. No *E. coli* strains were recovered from one subject; the number of isolates from the remaining subjects ranged from 2 to 38, with a mean of 18.

Multilocus enzyme electrophoresis

Aqueous protein extracts were prepared and run on 5% polyacrylamide gels in TBE buffer (Tris 0·089 M, boric acid 0·089 M, EDTA 0·002 M, pH 8·4) using the methods described by Selander and colleagues [6]. Eight enzymes were assayed for electrophoretic mobility; 6-phosphogluconate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, β-galactosidase, phosphoglucose isomerase, glutamic-oxalacetic transaminase and superoxide dismutase.

Plasmid analysis

The method of Bennett and colleagues [7] was used. Organisms were lysed using lysozyme and alkaline sodium dodecyl sulphate and the lysate was extracted with phenol. Plasmids were separated in 0·8% agarose gels with TBE buffer.

RESULTS

Proportion of polymorphic enzymes and number of alleles

All eight enzymes were polymorphic, the number of alleles (including the null phenotype) ranging from four for malate dehydrogenase, glucose-6-phosphate dehydrogenase, alcohol dehydrogenase and glutamic oxalacetic transaminase to seven for 6-phosphogluconate dehydrogenase.

Number of electrophoretic types and their distribution in subjects and in time

All the 269 isolates studied could be electropherotyped; 60 unique combinations of alleles, or electrophoretic types (ETs) were identified. Of these ETs, 81% were each recovered from only one subject (Table 1), and 50% were isolated only once

during the study period. Of the ETs recovered more than once from only one subject, 6 were isolated twice, 4 were isolated 3 times, 2 were isolated 4, 5 and 7 times respectively, and single ETs were isolated 6, 9, 14 and 15 times respectively.

The number of ETs recovered from the 13 subjects from which 10 or more isolates were made during the study period ranged from 2 to 8, with a mean of 5·8 (Table 1).

In eight subjects (1, 2, 3, 5, 6, 8, 13 and 16) 50% or more of isolates belonged to a single ET. In three of these subjects (1, 5 and 13) a second ET accounted for more than 25% of the remaining isolates.

In one subject (15) two ETs accounted for 37% and 31% of isolates respectively: these ETs differed at only one locus, ADH being null in ET 55 and having a mobility of 1 in ET 52. Both ETs had identical plasmid profiles (eight bands) and it is possible that the difference between them is artifactual.

New ETs were isolated at every sampling point during the study; at the last of these 6 new ETs were isolated from 5 subjects. Transient ETs which were only isolated once also occurred throughout the study, 4 of the 30 ETs in this category being isolated in its last week, after the end of the isolation period.

Plasmid analysis of ETs

Characterization of plasmids by number and molecular size revealed 30 different profiles, their type ranging from those characterized by the presence of a single band on gels to one having a complex pattern of 10 bands. Strains belonging to 12 ETs contained no detectable plasmids. Nineteen ETs had unique plasmid profiles; of the remainder, 5 pairs, 3 groups of 3 and 1 group of 6 ETs had indistinguishable plasmid profiles.

Strains of only one ET, 11, could be differentiated by plasmid type, some lacking plasmids, some possessing 2 plasmids > 15 kB and others possessing 5·3 kB and 4·2 kB plasmids.

Occurrence of electrophoretic types in more than one subject

Eleven electrophoretic types were found in more than one subject (Table 2). Five occurred sporadically during the study, being isolated on single occasions from 2 subjects (3 ETs) or 3 subjects (2 ETs).

Four ETs (6, 9, 10, 45) were repeatedly isolated over long periods from a single subject, with single isolations being made from other subjects. Only one of these (ET 6) was isolated first in a subject in which it did not predominate. This ET was isolated on one occasion from subject 9, in week 4. After a 12-week gap it was then isolated from subject 1, from which it was then obtained repeatedly. Prior to the isolation of ET 6, ET 3 had predominated in this subject, being isolated in weeks 6, 7 and 9 but not thereafter.

Two ETs (11, 28) were isolated repeatedly from more than one subject. ET 11 was isolated repeatedly from subject 6 at the beginning of the study and in its penultimate weeks, with a range of other ETs occurring in the intervening period. A similar temporal pattern was seen in subject 3, with single isolations being made at the two extremes of the study, bracketing the isolatiuon of 6 other ETs, ET 10 predominating. ET 11 was also isolated from three other subjects in week 10 (subject 14), week 28 (subject 9) and week 30 (subject 15). ET 28 was isolated

Table 1. *Distribution of electrophoretic types of* E. coli *amongst the subjects*

	Subject number														
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16
No. of samples...	32	16	27	2	14	26	13	18	11	4	11	38	10	19	28
ETs unique to subject	1	—	12	17	19	21	31	32	36	40	41	46	48	52	57
	2	—	13	18	20	22	—	33	37	—	42	47	49	53	58
	3		15			23		34	38		44		50	54	59
	4		16			24		35	39					55	60
	5					25								56	
	7					26									
	8														
ETs occurring in more than one subject	6	9	10	—	11	11	10	29	6	9	11	45	11	11	28
		10	11			27	14		11	11	28		27	45	43
			14				27		30		29		51	51	
							28				43				
							29								
							30								
Predominant ETs	3	9	10		19	11	29	33			45			52	28
	6				20						47	55			

Table 2. *Temporal distribution of the electrophoretic types of* E. coli *that occurred in more than one subject during the study period*

	Subjects with electrophoretic types identified as										
Week number	6	9	10	11†	11†	14	27	28	29	43	45
0	—	—	—	3	—	—	—	—	—	—	—
2				6				16. 12			
4	9*			6							
6			3	6							
8			3					7			
10		2. 11		14				16	8		13
12								16			
14			3								13. 15
16	1		3		12			16		12	
18	1	2	2								13
20		2	3					12			13
22											
24	1	2	3			3	7	16. 7		16	13
26			3		11	3. 7	14	16			13
28		2		9				12			13
30	1	2	3	33. 6. 15			6	16	7. 12		13
32	1		7	6				16			13

* Subject number.
† Distinguished by plasmid profile.

repeatedly during the study from three subjects, being the predominant ET in subject 16 and the commonest ET in subject 12.

DISCUSSION

The first studies on the microbial flora of residents of Antarctic bases were conducted during the 'heroic age' of exploration and were largely concerned with the effects of climatic extremes and prolonged residence in areas with no naturally

https://doi.org/10.1017/S0950268800049232 Published online by Cambridge University Press

occurring resident pathogenic prokaryotes [8]. Recent work has continued to emphasize the latter feature, particularly the unbroken isolation of small groups of individuals for more than 6 months of the year. The advantages of this circumstance for studies on the dynamics of carriage of commensals and their transmission are self-evident.

Previous studies on temporal variations in the population structure of *E. coli* in the human gut as measured by serotype have shown that certain strains persist for weeks or months, whereas others are transient, being present for only days or a few weeks [9]. A detailed study of the allozyme types occurring over an 11-month period in a single individual gave similar findings. Fifty-three electrophoretic types were isolated, 2 being resident, 1 recurrent and 50 transient [2]. It was concluded that the genotypic diversity was caused by immigration of bacteria from environmental sources. A similar pattern was demonstrated in the present study, in which half of the subjects were shown to have one or two dominant ETs. These ETs accounted for 50% or more of isolates. Transient ETs were isolated frequently from most of these subjects, each usually on only one or two occasions. Three other less frequent patterns also occurred. The flora of one subject (2) was dominated by a single ET (15 isolates), with only one transient being isolated. Subject 13 also provided very few transient isolates (two of the same ET) and his flora was dominated by two ETs (45, 21 isolates; 47, 15 isolates). Three subjects had floras composed only of transients.

Two ETs (11 and 28) each occurred in several subjects, and it is possible that these are examples of those *E. coli* clones which are geographically widespread and which have persisted in stable form for several decades [10, 11]. The subdivision of ET 11 into three distinct types by plasmid profile lends support to this hypothesis.

Despite the uninterrupted isolation of subjects during the study period and their consumption of only preserved and frozen food, a very diverse *E. coli* population was maintained by base members, with new and transient ETs being isolated throughout the study. The source of these transient ETs was not established. The possibility that they arise by recombination has already been shown to be very unlikely [2]. Their sporadic occurrence does not provide evidence favouring either of the two alternatives of person-to-person spread or acquisition from food, and the possibility cannot be ruled out that the full diversity of resident ETs was not revealed by the sampling protocol that was used. Further studies in which a greater number of strains are isolated from individuals at each sampling point will be required to distinguish between these alternatives. However, the sharing of some ETs among hosts, and the pattern of sharing, with 4 of the 11 shared ETs being predominant in a single host with single isolations being made from other hosts, provides *prima facie* evidence of spread of these ETs from subject to subject.

ACKNOWLEDGEMENTS

This work was supported by the British Antarctic Survey.

REFERENCES

1. Selander RK, Caugant DA, Whittam TS. Genetic structure and variation in natural populations of *Escherichia coli*. In: Neidhardt FC et al (eds). *Escherichia coli* and *Salmonella*

typhimurium cellular and molecular biology. Washington: American Society for Microbiology, 1987: 1625–48.
2. Caugant DA, Levin BR, Selander RK. Genetic diversity and temporal variation in the *E. coli* population of a human host. Genetics 1981; **98**: 467–90.
3. Caugant DA, Levin BR, Selander RK. Distribution of multilocus genotypes of *Escherichia coli* within and between host families. J Hyg 1984; **92**: 377–84.
4. Whittam TS, Wolfe ML, Wilson RA. Genetic relationships among *Escherichia coli* isolates causing urinary tract infections in humans and animals. Epidemiol Infect 1989; **102**: 37–46.
5. Ochman H, Whittam TS, Caugant DA, Selander RK. Enzyme polymorphism and genetic population structure in *Escherichia coli* and *Shigella*. J Gen Microbiol 1983; **129**: 2715–26.
6. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986; **55**: 873–84.
7. Bennett PM, Heritage J, Hawkey PM. An ultra-rapid method for the study of antibiotic resistance plasmids. J Antimicrob Chemother 1986; **18**: 421–4.
8. McLean AL. Bacteriological and other researches. Australasian Antarctic Expedition 1911–1914. Scientific Reports, Sydney 1918; **7** part 4: 107–14.
9. Cooke EM. *Escherichia coli* and man. Edinburgh: Churchill Livingstone, 1974.
10. Achtman M, Mercer A, Kusecek B et al. Six widespread bacterial clones among *Escherichia coli* K1 isolates. Infect Immun 1983; **39**: 315–35.
11. Ochman H, Selander RK. Evidence for clonal population structure in *Escherichia coli*. Proc Natn Acad Sci USA 1984; **81**: 198–201.