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The effect of travel on faecal *Escherichia coli* serotypes

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SUMMARY

In a study on the serotypes of *Escherichia coli* which are present in the faeces of students travelling from many parts of the world to Dublin, a great variety of types was found. It was not possible to relate certain types to the various parts of the world where the students came from. There was no decrease in variety of serotypes after the students had been in Dublin for a few months and no distinctive 'Dublin' types were acquired. Serological variation of the *E. coli* was suggested as partially explaining some of the variety of the serotypes found. Although eight students developed diarrhoea without an aetiological agent being isolated after arrival in Dublin, no distinct pattern of *E. coli* could be related to this.

INTRODUCTION

Since the studies of Kauffmann (Kauffmann, 1947) laid the foundations for the internationally accepted serotyping scheme of *Escherichia coli* strains, there have been many reports on the use of this antigenic scheme to study strains from a variety of sources from many parts of the world. These strains have generally been derived from various disease conditions such as gastroenteritis and urinary tract infections rather than from the faeces of healthy individuals. It has generally been assumed that the strains causing urinary tract infections are derived from the normal faecal flora of the patients, therefore any geographical variation found in these strains (Grüneberg & Bettelheim, 1969; Wong & Bettelheim, 1976) merely reflects a similar geographical variation in faecal serotypes. Studies on travellers' diarrhoea (Rowe, Taylor & Bettelheim, 1970) have suggested that this may be due to the acquisition of serotypes of *E. coli* not found in the country of origin of the traveller. Thus in order to gain an understanding of the role of faecal *E. coli* in travellers' diarrhoea it is important to gain an understanding of the geographical distribution of *E. coli* serotypes. However, owing to the large numbers of *E. coli* serotypes which can be found in a single specimen of faeces

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Table 1. *Countries of origin of the students*

Country of origin	No. of students studied in 1971	1972	1973	Total no. of students
Norway	14	12	8	34
U.S.A.	1	2	6	9
Canada	3	4	1	8
Mauritius	3	—	1	4
Hong Kong	—	1	2	3
Sweden	1	1	1	3
Trinidad	—	1	1	2
Ethiopia	—	1	—	1
Ghana	1	—	—	1
Greece	—	1	—	1
Iraq	1	—	—	1
Israel	1	—	—	1
Kenya	—	—	1	1
Malaysia	—	1	—	1
South Africa	—	1	—	1
Total	25	25	21	71

(Bettelheim, Faiers & Shooter, 1972) any such attempt by the current methods available is bound to raise many difficulties.

The study reported here, based on students coming from overseas to Dublin, was undertaken to determine the role of *E. coli* in the production of travellers' diarrhoea (this diarrhoea presumably would develop shortly after arrival or at least within 2 months of taking up residence in this or any other country): to find out if newly-acquired serotypes were similar, indicating a particular geographical pattern of 'Dublin' types, and to study the stability of serotypes in the students after some weeks/months residence in Dublin.

MATERIALS AND METHODS

The subjects

Five hundred and ninety-one specimens of faeces were obtained from 71 students from various parts of the world (Table 1) who travelled by air in the summers of 1971, 1972 and 1973, before and after they arrived in Dublin (Table 4). Following instructions sent to the students, they placed their pre-arrival specimens in Stuart's transport medium and posted them by airmail to Dublin. During the first 4 months of their stay in Dublin, specimens were brought by them each week to the laboratory. The development of diarrhoea by any student during the period of study was noted.

The specimens

All specimens were cultured on MacConkey agar No. 3 and, after 18–24 h incubation at 37 °C, generally up to ten colonies resembling *E. coli* were picked, attempts being made to include as many colonial variants as possible. Strains were considered *E. coli* on the basis of the methods of Cooke, Ewins & Shooter (1969).

Table 2. *O and H typability of strains isolated from a total of* 591 *specimens from* 71 *students*

Type of strain	Number
O typable	2874
O not typable	1260
Rough	937
H typable	3404
H not typable (but motile)	303
Not motile	1364
Total	5071

Serotyping methods

The strains of *E. coli* were 'O' serotyped according to the methods of Bettelheim *et al.* (1975) using 158 standard 'O' sera and 'H' serotyped by the methods of Chandler & Bettelheim (1974) using 53 standard 'H' sera. Strains which were autoagglutinable in saline were considered rough and designated 'R'. Strains which did not react at all or only at low titres with any of the standard 'O' or 'H' sera were considered non-typable and designated 'Ont' or 'Hnt'. Strains which remained non-motile by microscopic observation after repeated subculture in semi-solid medium in Craigie tubes were considered non-motile and designated 'H−'.

RESULTS

The 591 faecal specimens yielded 5071 strains of *E. coli*. Approximately 60% of these could be serotyped with available antisera. The number of O and H typable and non-typable strains is listed in Table 2. In all 374 different 'OH' serotypes were identified and the majority of these were found only in individual students. Only 90 were found in more than one student. Those serotypes from which the 'O' antigen could be identified and which were found in four or more students are listed in Table 3.

In order to assess the effect of travel and taking up residence in another country on the faecal *E. coli* of the students, the numbers of different serotypes found before the students left their country are compared with those found in the first and second 2 months of their stay in Dublin (Table 4). The largest group was from Norway and there was no similarity in their serotypes before arrival.

The general tendency observed with the faecal *E. coli* was that there was very little overlap of serotypes from one specimen to the next. Thus as an example student 5 yielded in the first specimen taken in Norway (22. vii. 1971) the following serotypes: O19.H7, O19.Hnt, O145.H33. Just before leaving he still excreted O19.H7 but also O19.H10 and O115.H10. The serotypes found in his faeces on the next three times when specimens were taken in Dublin were as follows: on 10. ix. 1971: O115.H10, O152.H8, R.H10, R.Hnt, and R.H−; on 15. ix. 1971: O1.H7 and O1.H33; on 20. ix. 1971: O1.H7, O1.H−, Ont.H4 and Ont.H7. Serotypes O1.H− and O19.H7 were found intermittently during November or December.

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Table 3. Escherichia coli *serotypes isolated from four or more students*

E. coli serotypes	No. of students from whom isolated
O1.H7	6
O1.H42	4
O2.H4	5
O2.H5	4
O6.H1	8
O6.H4	4
O18ab.H14	8
O21.H4	6
O21.H12	4
O29.H4	5
O75.H2	4
O75.H55	8
O83.H4	4
O117.H27	4
O124.H25	6
O147.H14	5
O148.H30	7
O154.H17	4
O1.Hnt	4
O2.Hnt	8
O11.Hnt	4
O1.H−	13
O2.H−	10
O6.H	6
O8.H−	5
O15.H−	4
O20.H−	4
O21.H−	5
O75.H−	5
O77.H−	4
O106.H−	4
O147.H−	4
O148.H−	8

Of the 71 students studied, only 63 co-operated fully throughout the periods of study and of these, nine developed diarrhoea after arriving in Dublin. *Shigella sonnei* was isolated from the faeces of one of these students but from the remaining eight no known enteropathogenic bacteria were isolated. The general tendency of change in faecal serotypes was observed in the periods of diarrhoea. The newly acquired *E. coli* serotypes found during the diarrhoeal stages are listed in Table 5.

DISCUSSION

The number of serotypes found in this study to be associated with the faeces of these students confirms previous work (Bettelheim *et al.* 1972) on the variety of serotypes normally present in faeces. This makes a study of this kind extremely difficult to interpret. However, a number of general conclusions can be drawn from these studies.

Table 4. *Number of different serotypes of* E. coli *found in faeces of students before arrival in Dublin, during first 2 months and second 2 months in Dublin*

No. of different *E. coli* serotypes found before students left for Dublin (A), during first 2 months (B), and second 2 months (C) in Dublin

Origin of students	No. of students	O typable									O not typable									O antigen, rough									Total		
		H typable			H not typable			Not motile			H typable			H not typable			Not motile			H typable			H not typable			Not motile					
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Norway 1971	14	27	70	51	6	9	9	8	32	16	10	22	14	1	1	1	1	1	1	7	14	10	1	1	1	1	1	1	62	151	104
Norway 1972	12	23	45	15	2	7	2	7	6	8	6	9	9	1	1	1	1	1	1	8	16	8	1	1	1	1	1	1	50	87	46
Norway 1973	8	16	37	32	0	2	0	1	6	8	5	11	10	1	0	1	1	1	1	6	10	11	1	1	1	1	1	1	32	69	65
U.S.A.	9	22	35	15	2	4	2	0	8	4	6	13	7	0	1	1	1	1	1	3	7	7	0	1	1	1	1	1	35	71	39
Canada	8	10	27	16	0	4	2	2	11	6	5	12	4	0	1	0	1	1	1	0	5	7	0	1	1	0	1	1	18	63	38
Africa*	4	2	5	10	1	0	2	3	4	3	6	5	6	0	1	1	0	1	1	2	6	4	0	1	0	1	1	1	15	24	28
Mauritius	4	5	19	13	0	1	2	5	5	3	1	6	3	0	0	1	1	1	1	2	4	2	0	1	0	1	1	1	15	38	26
Sweden	3	6	8	7	2	0	0	6	5	2	3	11	1	1	1	1	1	1	0	4	4	0	0	0	0	1	1	1	24	31	12
Eastern Mediterranean†	3	5	13	4	1	1	1	0	4	5	9	6	7	1	0	0	1	1	1	5	2	2	1	0	0	1	1	1	24	28	21
Trinidad	2	5	1	5	0	0	0	0	1	1	3	1	2	0	0	0	0	0	1	3	0	0	0	0	0	0	0	1	11	3	10
East Asia‡	4	10	9	11	1	0	0	3	2	1	6	3	6	0	1	1	1	1	1	5	3	7	0	0	0	0	1	1	26	20	28

* 'Africa' includes students from Ethiopia, Ghana, Kenya and South Africa.
† 'Eastern Mediterranean' includes students from Greece, Iraq and Israel.
‡ 'East Asia' includes students from Hong Kong and Malaysia.

Table 5. *Serotypes of* E. coli *newly acquired by students developing diarrhoea*

Student no.	Time of developing diarrhoea after arrival in Dublin (weeks)	Serotypes of *E. coli* isolated during diarrhoeal phases which were not present before: Serotypes only found during diarrhoeal phase	No. of other serotypes
4	1	—	4
14	2	O20.H38; O46.H−; O75.H21	7
18	2, 6 } 2 episodes	—	1
		O83.H31	1
24	1	O68.H12; O101.H9; O101.H18	7
56	1	—	2
61	7	—	0
67	7	O39.H7	3
69	1	O16.H−	6

It might be expected that after the students had lived in one place for 4 months, the number of different serotypes would have become less. The fact that this was not observed may be due to the period of observation not being long enough for a stable *E. coli* flora to have established itself. Another reason may be that the habits of the students, eating in university canteens, restaurants, etc. were not conducive to the development of a stable intestinal *E. coli* flora, as was found in a survey on one group of people eating mainly at home (Shooter *et al.* 1977). In their investigation, one person only had a wide variety of serotypes and he ate also in a canteen.

It is presumed that these changes in dietary habits will cause a constant new flow of *E. coli* to be ingested. Some of these may implant and others may just pass through. It seems likely that this would produce a totally random variety of serotypes. Although some randomness was observed, it was noted in virtually every student that the serotypes could often be grouped into families of related groups sharing one of the antigens studied. The student No. 5 described as an example in the Results section, yielded just such a related group of serotypes: O19.H7; O19.Hnt; O19.H10; O115.H10; R.H10; O1.H7; O1.H33; O1.H− and Ont.H7. Families of this kind occurred in all cases and it seems unlikely with the large variety of serotypes which were found in all the students, that this could be due just to coincidence. It is possible that serotypes carrying certain antigens are more likely to implant in the host as a result of some adhesive properties of the host intestinal mucosal epithelium. However, serological variation of the type observed previously (O'Farrell & Bettelheim, 1976) may be more widespread in the environment than had been considered. Thus in the example quoted above it is possible that the O19 strains might be able to 'switch' from carrying one H antigen to another and thus exhibit a phenomenon like phase variation observed for many years in the *Salmonella* group. Only extensive genetic and serological studies on such a family of strains could clarify this situation.

The development of diarrhoea by a small group of the students could not be correlated with acquisition of any particular pattern of serotypes (Table 5).

In recent studies Ørskov *et al.* (1976) suggested that enterotoxigenicity is limited to a certain small number of O:H serotypes among over 20000 which had been studied by them. None of the serotypes listed in Table 5 belong to this group. It is therefore not possible to assess their role in the diarrhoeal diseases of the students. Also as some of the students did not appear to acquire new serotypes, the role of *E. coli* in their cases cannot be assessed. The only way in which this could have been solved would have been to have tested for enterotoxin production, but as a very large number of strains were involved and there seems at present no absolutely reliable method of testing for enterotoxigenicity among *E. coli*, it was decided not to carry out these determinations.

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