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The biotyping of *Escherichia coli* isolated from healthy farm animals

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SUMMARY

A total of 2973 *Escherichia coli*, isolated from six different groups of animals, were examined for their ability to ferment adonitol, dulcitol, raffinose, rhamnose and sorbose in solid media. Twenty-nine fermentation patterns were recorded although 2443 (82%) of the *E. coli* belonged to seven of the 32 possible biotypes. Ninety-six O-serotypes were identified within the 2973 *E. coli*. The number of O-serotypes represented in the 15 most common biotypes ranged from three to 15. Serotypes O 8 and O 9 were found most commonly in the different groups of animals and several biotypes amongst these two O-serotypes were identified in two or more groups of the animals. The ability of the *E. coli* to metabolize aesculin, ornithine, salicin and sucrose was also assessed. These tests proved less reproducible and were not included in the primary biotyping scheme although their use allowed the enumeration of additional biotypes. The application of biotyping to the study of the ecology of drug-resistant strains of *E. coli* in five situations is briefly presented.

INTRODUCTION

Biological variation has been exploited for taxonomic purposes in that traits commonly present or absent are of value for identification of different species while those showing variable expression may be employed for the differentiation of strains within a species.

A number of schemes have been reported for differentiating (biotyping) strains of *Escherichia coli* isolated from human patients (particularly those suffering from urinary infections) based on the investigation of their antigenic properties, metabolic activity, morphology and resistance to either antibacterial drugs or chemicals (Bettleheim & Taylor, 1969; Elek & Hignay, 1970; Davies, 1977; Buckwold *et al.* 1979; Crichton & Old, 1979; Old *et al.* 1980.)

One typing method alone is often not satisfactory for confirming the identity

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of strains, but the use of methods in combination makes identification more accurate (Old *et al.* 1980). In our studies on the ecology of *E. coli* we have relied heavily on O-serotyping and antibacterial drug resistograms as tools for general survey purposes, but often it has not been possible to be certain that isolates, similar in all respects by these two methods of typing, are identical. In order to help overcome this problem a simple biotyping scheme was developed for use in parallel with the other typing methods.

Selection of substrates for evaluation

Our studies frequently involve the examination of relatively large numbers of epidemiologically related strains of *E. coli*, and consequently it is essential that any biotyping scheme be simple, highly reproducible, relatively cheap and applicable for use with a multi-pronged inoculator, since this is now used routinely in this laboratory for the *in vitro* testing of resistance to antibacterial drugs.

Edwards & Ewing (1972) and others have recorded the variability of *E. coli* strains to metabolize certain carbohydrates and amino acids. A review of literature (Table 1) suggests that carbohydrates suitable for consideration in a biotyping scheme include sorbose, rhamnose, mellibiose, sucrose, raffinose, adonitol, dulcitol, glycerol, aesculin and salicin. The ability to metabolize certain amino acids, in particular ornithine, also discriminates between strains of *E. coli*, although these tests have been found to be poorly reproducible, and this makes them a less suitable choice for biotyping purposes (Bettleheim & Taylor, 1969).

In making the selection of substrates for evaluation glycerol and arginine were omitted since they were considered by Crichton & Old (1979) to be of little value; mellibiose and lysine were also left out as we found these to be insufficiently discriminatory in preliminary studies (Table 1). On the other hand ornithine decarboxylation was included initially since this test has been employed by others for biotyping purposes (Davies, 1977; Crichton & Old, 1979).

MATERIALS AND METHODS

A total of 2973 isolates of *Escherichia coli* were examined. They were isolated from swabs of rectal or cloacal faeces obtained from healthy animals of different species including ten dairy cows maintained on the University Farm, Langford (40 isolates); fourteen dairy calves reared on the University Farm, Langford (879 isolates); four veal calves reared on a farm in Shipham, Somerset (580 isolates) – the calves were swabbed eight times during the first 14 days that they were on the farm; a sow and her litter of piglets reared on a farm in Winscombe, Avon (361 isolates); eight laying hens maintained in battery cages by the Department of Animal Husbandry, Langford (90 isolates) and a group of 100 broiler chicks in which the persistence of drug resistance in the faecal *E. coli* was studied (1023 isolates).

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Isolation and identification of E. coli

Lactose-fermenting *E. coli* were isolated initially on bile lactose agar, without salt (BLA, Oxoid CM 7b) following overnight incubation at 37 °C in air. Their identity was confirmed by a positive indole and Eijkman test and in some cases by a negative citrate test. The isolates were stored on nutrient agar slopes at room temperature until tested. All isolates were O-serotyped by a microtitre agglutination technique using 157 grouping sera (Howe & Linton, 1976). The resistance of the isolates from veal calves to antibacterial drugs was determined by a disk diffusion method (Linton, Howe & Osborne, 1975). The disks used included ampicillin (25 μg) (A), chloramphenicol (50 μg) (C), kanamycin (30 μg) (K), streptomycin (25 μg) (S), sulphafurazole (500 μg) (Su) and tetracycline (50 μg) (T).

Media used for biotyping

The carbohydrates used to differentiate fermentation properties included adonitol, dulcitol, D-raffinose, L-rhamnose, salicin, L-sorbose and sucrose. The media consisted of 1 % (w/v) carbohydrate, 1 % peptone, 0·5 % NaCl, 0·004 % phenol red and 2 % agar base. The pH was adjusted to 7·6 prior to autoclaving at 103 °C for 30 min.

Aesculin hydrolysis was assessed on media containing 0·1 % (w/v) aesculin, 0·05 % ferric citrate, 0·5 % NaCl, 1 % peptone and 2 % agar base. The method for the preparation of the ornithine medium is described by Cowan & Steele (1974). Sterile liquid paraffin was placed on the surface of the medium prior to incubation. Basal medium without ornithine served as a control.

Inoculation and incubation of media

Organisms from the nutrient agar slope were streaked onto a BLA plate and after overnight incubation a single colony was selected for testing. The plates were inoculated in one of two ways. In the first the colony was emulsified in 5 ml peptone water and the test media inoculated with a straight wire, while in the second a BLA master plate was inoculated initially and following 4 h incubation the carbohydrate media were inoculated from the masterplate with a multi-pronged inoculator (Denley Instruments A 400). Thirty-six strains were tested on a single 9 cm petri dish for each carbohydrate except aesculin where it was found to be more appropriate to test 20 strains on a plate. It was not found necessary to cut the agar between the inocula as suggested by Buckwold *et al.* (1979).

All media were incubated at 37 °C in air and the tests were read at either 24, 42 or 66 h as indicated under Results.

Interpretation of the test results

Acid production from adonitol, dulcitol, raffinose, rhamnose and sorbose was easily recognized by yellowing of the colony and the adjacent medium. The situation with salicin was sometimes more difficult as on occasions only part of the colony turned yellow. A blackening of the colony and surrounding medium was taken as evidence of aesculin hydrolysis. A black spot, developing only in the centre

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Table 1. *The metabolism of carbohydrate and amino acid substrates by* E. coli

Substrate	Proportion (%) metabolising the substrate										
	Ref 1*	2	3	4	5	6	7	8	9	10	11
Monosaccharides											
Arabinose	99	—	—	—	99	—	—	100	—	100	—
Galactose	—	—	99	—	—	—	—	—	—	—	—
Glucose	100	100	—	—	—	—	—	100	—	—	—
Rhamnose	82	92	87	—	89	—	94	99	88	87	80
Sorbose	—	—	—	66	—	—	—	—	—	58	42
Xylose	81	95	93	—	—	—	—	100	—	98	—
Disaccharides											
Cellobiose	3	8	—	9	—	—	—	—	—	1	—
Lactose	91	92	99	97	—	—	—	100	94	96	100
Maltose	90	99	100	97	—	—	—	100	—	96	97
Mellibiose	—	—	—	97	86	—	—	—	89	—	98
Sucrose	49	33	44	48	41	59	34	53	37	54	53
Trehalose	99	100	—	—	—	—	—	100	—	99	—
Trisaccharides											
Raffinose	51	—	32	54	—	39	41	68	24	57	53
Alcohols											
Adonitol	6	10	9	30	—	—	16	4	8	6	14
Dulcitol	50	64	60	48	—	89	50	84	—	69	38
Erythritol	0	—	—	—	—	—	—	—	—	—	—
Glycerol	89	—	84	—	—	—	—	—	—	—	—
Mannitol	97	100	—	—	99	—	—	98	—	—	—
Sorbitol	93	97	30	—	95	—	97	98	—	96	100
Glycosides											
Aesculin	31	—	—	—	—	—	—	—	—	52	29
Salicin	40	67	69	60	—	38	47	65	—	55	13
Amino acids											
Arginine	18	—	—	—	3	41	—	9	—	—	—
Lysine	89	98	—	88	69	—	—	100	92	91	99
Ornithine	64	46	—	67	71	85	—	74	—	62	84

*1. Edwards & Ewing (1972).
2. Møller (1954); 172 *E. coli*.
3. Pesti (1960); 400 *E. coli*.
4. Braatan and Meyers (1977); 33 *E. coli*.
5. Davies (1977); 574 *E. coli*.
6. Kulshrestha & Kumar (1978); 32 *E. coli*.
7. Magalhaes & Vance (1978); 32 H_2S positive *E. coli*.
8. Ishiguro *et al.* (1978); 80 citrate negative *E. coli*.
9. Buckwold *et al.* (1979); 959 *E. coli*.
10. Crichton & Old (1979); 917 *E. coli*.
11. Preliminary studies by the authors; between 500 and 800 *E. coli*.

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of the colony, was classed as negative. The decarboxylase test was read as positive when the medium became purple (alkaline) and the control medium remained yellow (acid).

RESULTS

The results are presented in three parts. The first describes the development of the biotyping scheme and is based on the examination of 580 *E. coli*, the second indicates the distribution of biotypes amongst 2973 *E. coli* isolated from several groups of animals, while the third describes briefly the application of biotyping in several specific situations.

The development of the biotyping scheme

The tests on 580 lactose-fermenting *E. coli* isolated from veal calves were performed in duplicate with the media being inoculated with a straight wire. Acid

Table 2. *The metabolism of nine substrates by 580* E. coli *isolated from veal calves*

Substrate	Result of duplicate tests		Test result at 24 h	Test result at 42 h	Test result at 66 h	Proportion (%) with both tests agreeing at 24 h	Proportion (%) with both tests agreeing at 42 h	Proportion (%) with both tests agreeing at 66 h	No. (%) additional positive tests recorded at 42 h	No. (%) additional positive tests recorded at 66 h
Aesculin	+	+	NR	118	148	NR	95·8	95·2	NR	24 (4·1)
	+	−		24	28					
	−	−		438	404					
Adonitol	+	+	79	79	NR	99·8	99·8	NR	0 (−)	NR
	+	−	1	1						
	−	−	500	500						
Dulcitol	+	+	274	278	NR	99·3	99·3	NR	4 (0·7)	NR
	+	−	4	4						
	−	−	302	298						
Ornithine	+	+	NR	471	475	NR	96·3	95·0	NR	12 (2·1)
	+	−		21	29					
	−	−		88	76					
Raffinose	+	+	301	304	NR	99·1	99·1	NR	3 (0·5)	NR
	+	−	5	5						
	−	−	274	271						
Rhamnose	+	+	450	460	NR	99·7	99·7	NR	10 (1·7)	NR
	+	−	2	2						
	−	−	128	118						
Salicin	+	+	NR	46	74	NR	95·2	95·9	NR	24 (4·1)
	+	−		28	24					
	−	−		506	482					
Sorbose	+	+	238	240	NR	99·5	99·5	NR	2 (0·3)	NR
	+	−	3	3						
	−	−	339	337						
Sucrose	+	+	224	250	NR	97·9	90·5	NR	68 (11·7)	NR
	+	−	13	55						
	−	−	243	275						

NR = not recorded.

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Table 3. *A scheme for biotyping* E. coli *based on acid production from five carbohydrates*

Biotype no.	Acid production from				
	Adonitol	Dulcitol	Raffinose	Rhamnose	Sorbose
1	+	+	+	+	+
2	+	+	+	+	−
3	+	+	+	−	+
4	+	+	−	+	+
5	+	−	+	+	+
6	−	+	+	+	+
7	+	+	+	−	−
8	+	+	−	+	−
9	+	+	−	−	+
10	+	−	+	+	−
11	+	−	+	−	+
12	+	−	−	+	+
13	−	+	+	+	−
14	−	+	+	−	+
15	−	+	−	+	+
16	−	−	+	+	+
17	+	+	−	−	−
18	+	−	+	−	−
19	+	−	−	+	−
20	+	−	−	−	+
21	−	+	+	−	−
22	−	+	−	+	−
23	−	+	−	−	+
24	−	−	+	+	−
25	−	−	+	−	+
26	−	−	−	+	+
27	+	−	−	−	−
28	−	+	−	−	−
29	−	−	+	−	−
30	−	−	−	+	−
31	−	−	−	−	+
32	−	−	−	−	−

production from adonitol, dulcitol, raffinose, rhamnose and sorbose was not recorded after 42 h since evidence of alkaline reversion was noted in a proportion of cases at 66 h. Tests for the metabolism of aesculin and salicin and ornithine decarboxylation were read at 42 and 66 h. The results for these reactions are listed in Table 2. Acid production from adonitol, dulcitol, raffinose, rhamnose and sorbose was highly reproducible, with over 99 % of the tests performed in duplicate giving the same result. In addition very few extra positive results were recorded after 42 h incubation. On the other hand the results for the other tests were less satisfactory although it was found subsequently that the reproducibility for sucrose increased considerably when the test was performed using the multi-pronged inoculator. On the basis of these findings it was decided to formulate a two-tier scheme for biotyping. The primary scheme involved the five carbohydrates, which

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Table 4. *The distribution of secondary biotypes amongst seven of the primary biotypes of* E. coli *isolated from veal calves*

	Secondary biotype*				Primary biotype†						
	A	O	Sal	Suc	6	13	15	19	24	30	32
a	+	+	+	+	1	—	—	2	—	1	—
b	+	+	+	−	—	—	—	21	—	16	1
c	+	+	−	+	16	2	—	3	3	—	1
d	+	−	+	+	—	—	—	—	—	—	—
e	−	+	+	+	—	—	—	—	—	—	—
f	+	+	−	−	5	3	1	8	2	2	2
g	+	−	+	−	—	1	—	3	1	1	—
h	+	−	−	+	1	2	—	3	—	—	1
i	−	+	+	−	—	—	1	—	—	2	1
j	−	+	−	+	84	50	8	2	38	3	1
k	−	−	+	+	—	—	—	—	—	—	—
l	+	−	−	−	—	—	—	16	—	2	9
m	−	+	−	−	8	1	12	8	22	10	13
n	−	−	+	−	—	—	—	1	—	—	—
o	−	−	−	+	2	—	3	—	2	—	6
p	−	−	−	−	—	—	16	1	1	8	7

* A, aesculin hydrolysis; O, ornithine decarboxylation; Sal and Suc = salicin and sucrose fermentation.

† See Table 3.

gave highly reproducible results when read at 24 h. These gave a maximum of 32 possible biotypes, which for the sake of convenience, have been assigned a number according to the sequence of test results (Table 3). The ability to metabolize aesculin, ornithine, salicin and sucrose was reserved for a secondary scheme with the types being designated by the letters a to p (Table 4). Their use provided additional discrimination between isolates since the seven most common primary biotypes were divisible into 56 primary/secondary biotype combinations (Table 4), although individual results have to be interpreted with caution since the secondary tests were relatively unreliable.

The distribution of biotypes amongst 2973 E. coli

The 2973 *E. coli* isolated from the six groups of animals were unevenly distributed amongst 29 of the 32 possible biotypes (Table 5). Biotypes 6 and 13 accounted for about 40 % of the isolates; 2443 (82 %) fell into seven biotypes (nos. 6, 13, 15, 19, 22 and 30).

Dulcitol (D), raffinose (Ra) and sorbose (S) were the most discriminatory of the five tests and divided the *E. coli* into eight fermentation patterns which contained between 91 (3 %) (D−, Ra+, S+) and 675 (23 %) (D+, Ra+, S−) of the 2973 isolates. The influence of the two less discriminatory tests, namely adonitol fermentation (A+) (11 % of the *E. coli*) and the failure to ferment rhamnose (Rh−) (11 % of the *E. coli*), was greater than might have been expected since the *E. coli* exhibiting these reactions were not evenly distributed amongst these eight

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Table 5. *The distribution of biotypes amonst* E. coli *isolated from various groups of healthy animals*

Biotype*	Total biotypes amongst *E. coli* isolated from: Dairy cows	Dairy calves	Veal calves	Sow and piglets	Laying hens	Broiler chicks	Total	Proportion (%)
1	—	2	—	—	—	3	5	—
2	—	3	—	—	—	2	5	—
4	—	—	—	—	—	1	1	—
5	—	1	—	—	—	—	1	—
6	9	106	117	48	64	323	667	22·4
8	—	—	1	—	—	1	2	—
10	—	—	3	—	—	23	26	0·9
11	—	—	—	—	—	1	1	—
12	—	—	3	—	—	2	5	—
13	14	286	59	55	6	172	592	19·9
14	—	—	—	—	—	3	3	—
15	—	10	41	1	19	201	272	9·1
16	1	13	27	—	—	28	69	2·3
17	—	1	1	—	—	—	2	—
18	1	2	—	—	—	—	3	—
19	5	105	68	21	—	19	218	7·3
20	—	1	—	—	—	1	2	—
21	—	—	2	—	—	5	7	—
22	3	88	28	—	—	54	173	5·8
23	—	2	18	—	—	—	20	0·7
24	3	46	69	182	—	41	341	11·8
25	—	44	32	—	—	2	78	2·6
26	—	23	1	21	—	31	76	2·6
27	1	12	4	29	—	—	46	1·5
28	2	52	15	—	—	16	85	2·9
29	—	—	—	—	1	17	18	0·6
30	1	63	45	4	—	67	180	6·1
31	—	3	4	—	—	1	8	—
32	—	16	42	—	—	9	67	2·3
Total	40	879	580	361	90	1023	2973	

* See Table 3.

fermentation patterns; 264 (83%) of the 317 A+ isolated were amongst the 511 D−, Ra−, S− strains while the majority (82%) of the Rh− *E. coli* were amongst those that were D−, Ra+, S+; D+, Ra−, S− and D−, Ra−, S−.

A total of 96 O-serotypes were identified within the 2973 *E. coli* and within these 25 primary biotypes were found (Table 6), while 14 non-typable *E, coli* represented biotypes 8, 14, 20 and 21.

Serotypes O8 and O9 were the most widely distributed of the O-serotypes amongst the different groups of animals. Seven of the biotypes of serotype O8 were present in more than one group of animals, with biotype 6 being found in five of the six groups. Similarly six of the 11 biotypes present in serotype O9 were identified in two or more groups of animals (Table 7).

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Table 6. *The number of O-serotypes identified within biotypes of* E. coli *isolated from various groups of healthy animals*

	No. of O-serotypes within each *E. coli* biotype						
Biotype*	Dairy cows	Dairy calves	Veal calves	Sow and piglets	Laying hens	Broiler chicks	All groups
1	—	1	—	—	—	2	3
2	—	3	—	—	—	2	5
4	—	—	—	—	—	1	1
5	—	1	—	—	—	—	1
6	5	17	9	6	3	11	30
10	—	—	1	—	—	2	3
11	—	—	—	—	—	1	1
12	—	—	2	—	—	—	2
13	7	35	5	8	2	12	52
15	—	4	8	1	—	11	19
16	1	3	7	—	—	7	14
17	—	1	—	—	—	—	1
18	1	1	—	—	—	—	1
19	3	16	10	1	—	3	25
22	2	17	3	—	—	9	26
23	—	2	4	—	—	—	6
24	—	9	5	5	—	6	19
25	—	4	4	—	—	—	7
26	—	3	1	2	—	6	12
27	1	4	—	2	—	—	7
28	1	8	3	—	—	—	10
29	—	—	—	—	1	1	1
30	1	17	9	2	—	9	33
31	—	1	3	—	—	1	5
32	—	2	3	—	—	1	5
Total O-serotypes	16	70	33	18	5	35	96

* See Table 3. No O-typable *E. coli* recorded amongst biotypes 8, 14, 20 and 21 (Table 5).

The application of biotyping to epidemiological studies

The data obtained for the 580 *E. coli* isolated from four veal calves, and on which the biotyping scheme was originally developed, were analysed further, and five examples are presented in which biotyping assisted in the interpretation of the information provided by O-serotyping and antibacterial drug resistogram typing.

(1) *E. coli* serotype O 123 was isolated on several occasions from one calf and the resistance patterns changed with time. During the first two days that the calf was on the farm the biotype was 32 and the resistance pattern SuT or SSuT. Later, between days 6 and 14, the serotype reappeared in the dominant faecal flora and carried R determinants to A, C and sometimes K in addition to SSuT. The biotypes of these isolates were nos. 6, 11, 24 and 25, and this suggests that the intestine had become colonized with new strains of the same O-serotype and that the change in the resistance patterns was not due to the acquisition of additional R plasmids by the original colonizing strain.

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Table 7. *The distribution of biotypes amongst* E. coli *O-serotypes 8 and 9 isolated from different groups of healthy animals*

O-serotype	Animal group	Biotype* 1	5	6	10	13	15	16	19	22	23	24	25	26	28	30	31
O8	Dairy cows	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−
	Dairy calves	−	+	+	−	+	−	−	−	+	−	−	+	−	+	+	−
	Veal calves	−	−	+	−	+	−	+	+	+	+	−	−	−	+	−	+
	Laying hens	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−
	Broiler chicks	−	−	+	−	+	+	−	+	+	−	−	−	+	−	+	−
O9	Dairy calves	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−
	Veal calves	−	−	−	−	−	+	+	+	−	−	+	−	−	−	+	−
	Broiler chicks	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−

* See Table 3; + indicates that the biotype was identified.

Table 8. *Distribution of secondary biotypes amongst biotypes of* E. coli *O-serotypes 8, 21, 86 and 118 isolated from four veal calves*

O-serotype	Biotype*	No. of isolates with secondary biotype† a	b	c	f	g	j	m	n	p	Total
8	6	—	—	4	3	—	7	3	—	—	17
	13	—	—	1	—	—	48	—	—	—	49
	16	—	—	—	—	—	4	—	—	—	4
	19	—	—	—	—	1	—	—	—	—	1
	22	—	1	—	1	—	2	—	—	—	4
	23	—	—	—	—	—	—	1	—	—	1
	28	—	—	—	—	—	—	1	—	—	1
	31	—	—	—	—	—	—	—	—	1	1
21	6	1	—	11	—	—	60	—	—	—	72
	19	—	3	—	—	1	—	—	1	—	5
	31	—	—	—	—	—	—	—	1	—	1
86	16	—	—	—	—	—	11	—	—	—	11
118	25	—	—	—	—	—	10	—	—	—	10

* See Table 3.
† See Table 4.

(2) Serotype O8 biotype 6j was isolated from all four calves while three biotypes (6c, 6m and 13j), which differed from 6j by only one of the nine test results, were isolated from two of the calves. The remaining biotypes were each found in one calf only (Table 8). These data indicate that a number of biochemical variants of an O-serotype, some of which are closely related biochemically and others which are not, may be found in the dominant faecal flora of one or more animals kept in close proximity within a relatively short space of time (two weeks).

(3) The situation regarding serotype O21, which was isolated from two calves, differed from the second example since the strains in the calves appeared to be dissimilar (Table 8). Biotype 6, the majority of which comprised the closely related types 6c and 6j, and which had the resistance patterns ACSu and ACSSu, was

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isolated from one calf together with biotype 31 (resistance pattern ACKSSuT) while another biotype (no. 19), which had the resistance patterns CKSSuT and ACKSSuT, was isolated from the second calf.

(4) Serotype O86 was isolated at one sampling occasion from one of the four calves. All 11 isolates were biotype 16j (resistance pattern KSu) suggesting that all isolates were derived from a single clone.

(5) Finally, serotype O118, biotype 25j was isolated from two calves on different sampling occasions; the resistance pattern in one calf was KSSu and in the other SSu (Table 8). The only difference was the presence of an R determinant for kanamycin, and so in this case additional characteristics would need to be examined to establish whether these isolates had a common identity or not.

DISCUSSION

The ideal test for inclusion in a biotyping scheme divides epidemiologically unrelated strains 50:50, although Crichton & Old (1979) suggested that 90:10 is probably the lowest acceptable level of discrimination. This proposal is supported by our observation that the fermentation of adonitol and the failure to ferment rhamnose, the two least discriminatory of the five tests in the primary biotyping scheme, were highly discriminatory within three of the eight fermentation patterns identified by the metabolism of dulcitol, raffinose and sorbose. As a consequence it is possible that other tests with apparently poor powers of discrimination, such as mellibiose and xylose fermentation (Table 1) may prove discriminatory in certain circumstances.

Similarly, the inclusion of both raffinose and sucrose in a primary biotyping scheme may also prove justified even though the majority of *E. coli* give the same result for both tests (Ørskov & Ørskov, 1973; Crichton & Old, 1979), since those *E. coli* that do not do so apparently elaborate different fermentative enzymes (Arr, Perenyi & Novak, 1970).

The tests in the primary biotyping scheme were read after a relatively short period of incubation and as the results were highly reproducible the tests were probably qualitative assays of constitutive enzymes. On the other hand tests which assay inducable enzymes, such as the beta-glycosidases which metabolise aesculin and salicin (Edberg, Pittman & Singer, 1977; Miskin & Edberg, 1978) tended to be unsatisfactory for biotyping since the definition of a satisfactory end-point was difficult (Table 2; Crichton & Old, 1979). However, induced aesculin hydrolysis does discriminate satisfactorily between *E. coli* strains, and if the test could be modified, possibly by employing pre-incubation in aesculin broth to give a clear end-point, it would prove worthy for inclusion in a primary biotyping scheme.

Most characters included in biotyping and resistotyping schemes are subject to variation with the spread of a clone, and this may be due to the acquisition or loss of a plasmid or phage which codes for that character (Crichton & Old, 1979). This must be taken into account when the tests are interpreted in an epidemiological context since the ability of *E. coli* to produce hydrogen sulphide or to metabolize raffinose and sucrose is transmissible in certain circumstances (Ørskov & Ørskov,

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1973; Smith & Parsell, 1975). Interestingly, *E. coli* which ferment raffinose by virtue of a transmissible *lac* permease gene do so only slowly (Cornelius, Luke & Richmond, 1978), and as a consequence, strains with this plasmid would probably have been classed as negative under the conditions of our test protocol and a correct assessment of the basic phenotype of the strain would have been made in those circumstances.

In the past we have employed O-serotyping as the basic tool for our studies of the ecology of drug resistance in *E. coli*. This technique is extremely time consuming and consequently it is essential that any additional discriminatory tests be economic in technician time. The biotyping of the *E. coli* using solid media and a multi-pronged inoculator fulfilled this criterion. The five tests selected for the primary biotyping scheme were highly reproducible and the results obtained prove helpful in assisting in the interpretation of our ecological data, five examples of which are referred to briefly in the results. However, we are mindful of the conclusion of Old *et al.* (1980) that the employment of several different discriminatory schemes enhances the differentiation between strains of *E. coli* and consequently the search for additional substrates suitable for inclusion in a primary biotyping scheme is at present in hand.

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