A BIOTYPING SCHEME FOR THE SUBSPECIFIC DISCRIMINATION OF *ESCHERICHIA COLI*

PAMELA B. CRICHTON AND D. C. OLD

*Department of Bacteriology, University of Dundee Medical School,
Ninewells Hospital, Dundee DD1 9SY*

SUMMARY. A two-tier system for biotyping *Escherichia coli* gave a fine and reliable differentiation of strains and is capable of future extension by the addition of new types and new tests. Strains were allocated to a primary biotype (1-16) by their reactions in four primary tests in culture media containing raffinose, sorbose, ornithine or dulcitol. Subtypes were distinguished within the primary biotypes by reactions in six secondary tests for rhamnose fermentation, lysine decarboxylation, aesculin hydrolysis, motility, type-1 fimbriation and prototrophy. Full biotypes were designated by letters indicating subtype reactions appended to primary type numbers.

A series of 599 strains (1242 cultures) of *E. coli* from diverse sources was classified into 16 primary biotypes and into 213 full biotypes. The biotype characters of a strain were generally stable during its spread in the natural environment and in non-selective media used for storage.

INTRODUCTION

Readily available and reliable methods for the subspecific typing of *Escherichia coli* are essential if the hospital laboratory is to help the epidemiologist in such matters as the detection of cross infection in surgical wards and nurseries and the clinician in the management of patients with urinary-tract infection. Although the complete antigenic analysis of *E. coli* affords excellent strain discrimination, the number of sera required is such that serotyping is most appropriate for use in reference laboratories. Accordingly, other techniques that allow characterisation of isolates of *E. coli* should prove invaluable in smaller laboratories in which there is a wish to study, independently of the use of serotyping, the epidemiology of *E. coli*.

We have demonstrated, for example, that biotyping of *E. coli* is highly discriminatory and that its biotypes are generally reproducible (Crichton and Old, 1979, 1980; Old *et al.*, 1980; Wilson, Crichton and Old, 1981). However, in these studies, we reported the results for clinical isolates of *E. coli* as biotype profiles, a method of designation that is cumbersome for routine purposes. In this communication we adopt the procedure successfully applied by Duguid *et al.* (1975) to *Salmonella typhimurium*, and propose a two-tier biotyping system of four primary and six

---

Received 1 Sep. 1981; accepted 29 Sep. 1981.

secondary tests for *E. coli*. The general usefulness of the scheme in identifying many different biotypes among a collection of *E. coli* strains and in defining accurately strains isolated from selected clinical cases is demonstrated.

MATERIALS AND METHODS

Bacteria. The 599 strains of *E. coli* examined (table I) included 177 O-serogroup strains from the National Collection of Type Cultures, London (NCTC), previously described by Crichton and Old (1979), and 422 strains from diverse clinical sources. The latter group of 422 strains, discriminated by multiple typing methods (Crichton and Old, 1980; Old *et al.*, 1980; Wilson *et al.*, 1981), was represented by the following 1065 cultures: 100 (WB) from Professor W. Brumfitt, The Royal Free Hospital, London, and 195 (MIW) from Dr Margaret I. Wilson, Department of Bacteriology, Ninewells Hospital, Dundee, from patients with persistent or recurrent urinary-tract infection; 770 (DU) isolated by the authors from wound swabs and various body fluids, tissues and aspirates received in the department of Bacteriology, Ninewells Hospital, Dundee, during 1976–1981, and identified as *E. coli* by conventional methods (Edwards and Ewing, 1972). Urinary isolates were from patients with significant bacteriuria, i.e., $> 10^5$ cfu/ml of freshly voided urine. The 1065 clinical isolates comprised series from 265 individual patients: 19 from neonates (N1–N19), 203 from female patients aged from 2 months to 92 years (F1–F203) and 43 from male patients aged from 1 month to 76 years (M1–M43). From some patients, only two cultures were obtained from specimens taken on the same day; from others, multiple specimens yielded isolates during periods of up to 3 years. Some of the series have been described previously (Crichton and Old, 1980; Old *et al.*, 1980; Wilson *et al.*, 1981). Isolates were stored on Dorset's egg slopes at ambient temperature until subcultured for testing.

Culture media. Nutrient Agar (pH 7.4) and Nutrient Broth (pH 7.5) were prepared according to the instructions of the manufacturer (Oxoid). All ingredients were dissolved in deionised water. Broth was dispensed in 10-ml volumes in cottonwool-stoppered test tubes (15 × 1.5 cm) and autoclaved. All agar media were poured in 20-ml amounts in plastic petri plates (8.5 cm in diameter).

Aesculin agar (pH 7.4) was prepared according to the method of Cowan (1974). Glucose-mineral salts agar contained K_2HPO_4 7 g, KH_2PO_4 3 g, $(NH_4)_2SO_4$ 1 g, $MgSO_4 \cdot 7H_2O$ 0.1 g and Difco Bacto-Agar 15 g/L (pH 7.0), with 0.3% (w/v) D-glucose. Motility medium containing Oxoid Tryptone Water 15 g and Difco Bacto-Agar 3 g/L, was dispensed, after thorough mixing, in 10-ml volumes in test tubes, as above, and autoclaved. Sugar peptone-water medium contained Oxoid Peptone Water 15 g and, as indicator, bromocresol purple 0.02 g/L with 0.5% (w/v) carbohydrates: dulcitol, D-raffinose, L-rhamnose, or L-sorbose. The sugar peptone media, dispensed in 4-ml amounts in sterile, screw-capped 6-ml bottles, were steamed at 100°C for 30 min. L-Lysine monohydrochloride or L-ornithine monohydrochloride (0.5% w/v) were added to decarboxylase basal medium containing Oxoid Bacteriological Peptone 5 g, Oxoid Yeast Extract 3 g, D-glucose 1 g, and bromocresol purple 0.02 g/L. Controls were basal medium without additions. The decarboxylase media, adjusted to pH 6.7, were dispensed in 5-ml volumes in screw-capped 6-ml bottles.

Except where otherwise stated, all culture media were autoclaved at 121°C for 15 min.

Inoculation of media. Half of a well isolated colony, picked from a pure culture of the test organism, was inoculated to a tube of nutrient broth, which was incubated statically for 48 h in air. The other half colony was spread on nutrient agar and the resulting growth, after incubation for 24 h, was suspended in physiological saline (NaCl 8.5 g/L) to a density of 10^9 cfu/ml. Biotyping media were inoculated with 0.02 ml of the suspension with either an inoculating wire (motility, aesculin and glucose-mineral salts media) or a capillary pipette (all other media). All tests were incubated at 37°C.

Interpretation of results. Fermentation tests were examined for the production of acid (indicator change from purple to yellow) after periods of incubation that differed according to the test substrate: sorbose and rhamnose tests at 24 h; dulcitol at 48 h; raffinose at 72 h. The definitive times of reading were selected to give optimal separation of genotypically hydrolysing from non-hydrolysing types. Decarboxylase tests were read as positive when the medium

became purple (alkaline) at 48 h and the basal medium without amino acid remained yellow (acidic). Plate tests for aesculin hydrolysis were examined at 72 h for growth with brownish-black papillae and a surrounding brownish-black precipitate. Glucose-mineral salts medium was examined for the presence or absence of growth after 24 h and growth requirements of auxotrophic strains were determined by the methods of Meynell and Meynell (1965). Results in motility medium were recorded as positive when growth had spread uniformly from the central stab inoculum at 24 h; any other result for that test was recorded as negative. Tests for the production of type-1 fimbriae (*fim*), as indicated by the presence of mannose-sensitive haemagglutination, were made with the centrifuged deposit of ten 48-h serial broth cultures by previously published methods (Duguid *et al.*, 1955).

RESULTS

Primary biotyping tests

In the proposed biotyping scheme, tests with raffinose, sorbose, ornithine and dulcitol were selected for the primary differentiation of *E. coli* on the basis of their reliability and discriminating power. Read at their definitive times, these tests discriminated 599 strains (1242 cultures) of *E. coli* almost optimally, i.e., close to 50% positive, 50% negative (table I), with slightly different proportions of positive types

TABLE I
Biotyping results for 599 strains* of Escherichia coli

Biotyping test (and character)	Percentage of strains positive from indicated source (number examined)				
	NCTC (177)	WB (69)	MIW (103)	DU (250)	All (599)
Fermentation of raffinose (raf)	47	55	54	54	52
Fermentation of sorbose (sor)	31	71	57	66	54
Decarboxylation of ornithine (orn)	41	67	71	64	59
Fermentation of dulcitol (dul)	61	72	66	71	67
Fermentation of rhamnose (rha)	82	93	92	87	87
Decarboxylation of lysine (lys)	88	88	92	92	90
Hydrolysis of aesculin (aes)	51	41	50	41	45
Motility (mot)	66	48	42	58	56
Production of type-1 fimbriae (<i>fim</i>)	76	94	84	85	83
Prototrophy (pro)	88	81	91	89	88

* When more than one isolate of a strain was available, the biotype of the first isolate was used in the calculations.

For explanation of abbreviation of sources, see *Materials and methods*.

noted among strains from the different collections examined; see, for example, results of tests with sorbose and ornithine. According to the different combinations of positive and negative reactions in the four primary tests, 16 primary biotypes were established (table II).

Secondary biotyping tests

Tests for motility and aesculin hydrolysis also afforded almost optimal type differentiation among the 599 strains (table I), but because their interpretation

TABLE II
Reactions of 16 primary biotypes of *Escherichia coli*

Primary biotype no.	Results of biotype test* with			
	raf	sor	orn	dul
1	+	+	+	+
2	+	+	+	-
3	+	+	-	+
4	+	+	-	-
5	+	-	+	+
6	+	-	+	-
7	+	-	-	+
8	+	-	-	-
9	-	+	+	+
10	-	+	+	-
11	-	+	-	+
12	-	+	-	-
13	-	-	+	+
14	-	-	+	-
15	-	-	-	+
16	-	-	-	-

+ = Positive, - = negative result.

* For explanation of biotype-test characters, see table I.

presented some difficulties (Crichton and Old, 1980), they were considered more suitable for use at the secondary level. Also used as secondary biotyping tests were those that, though aiding strain discrimination, differentiated less than 15% of the minority type. These tests (table I) were for rhamnose fermentation (13% minority type), lysine decarboxylation (10%) and growth-factor requirements (12%).

Most (88%) strains were prototrophic and grew well on glucose-mineral salts medium in 24 h. Only 74 of the 599 strains were auxotrophic and required supplementation of the mineral-salts medium with growth factors: 31 required nicotinamide (1 phenylalanine also; 1 thiamine also); 15 thiamine; 9 methionine (1 tryptophane also; 1 lysine also); 4 pantothenic acid (1 tryptophane also); 4 histidine; 3 proline; 1 tryptophane; 1 cysteine; 1 arginine; 1 leucine; and four required three or more undetermined growth factors. Three of the 74 auxotrophic strains were originally prototrophic but had become auxotrophic by the time of their second or subsequent isolation. Because the requirements for nicotinamide (42% of auxotrophs) and thiamine (20%) were relatively common, their determination was indicated in the biotyping scheme by specific characters (x and y, respectively); other, less common growth-factor requirements were indicated by the collective subtype character of z (table III).

Strains cultured serially in aerobic, static broth at 37°C were tested for the production of mannose-sensitive haemagglutinin, indicative of the presence of type-1 fimbriae (Duguid *et al.*, 1955). In *E. coli*, type-1 fimbriation, controlled by the chromosomal gene *fim* (Maccacaro and Hayes, 1961), is a relatively stable property which we have previously used as an adjunct to biotyping (Crichton and Old, 1980; Old *et al.*, 1980; Wilson *et al.*, 1981). The haemagglutination test indicated that a minority (17%) of the 599 strains examined did not produce type-1 fimbriae (table I), and this biotype character was assessed for use only at the secondary level.

TABLE III
Designation of results in secondary biotyping tests

Designation	Result
a	Positive results in all secondary tests
b	L-Rhamnose not fermented in 24 h
c	L-Lysine not decarboxylated in 48 h
d	Aesculin not hydrolysed in 72 h
e	Non-motile in 24 h, or motile variants produced after 24 h
f	Type-1 fimbriae not produced
x	Nicotinamide required for growth on minimal medium
y	Thiamine required for growth on minimal medium
z	A growth factor other than nicotinamide or thiamine required

The proposed subtype designations for each of the six secondary biotyping tests are explained (table III).

Discrimination provided by biotyping

The 599 strains, fully examined for all biotype characters, included representatives of all primary biotypes (table IV), and the results of the secondary tests differentiated these 16 primary types further into 213 full biotypes (table IV). The largest number of strains was found in primary biotype 1 (18%); these 110 strains were assigned to 23 full biotypes by means of results from the six secondary tests. The full biotype detected most frequently, biotype 1de, contained 29 strains.

Reliability of biotyping

When the complete scheme of four primary and six secondary tests was used to characterise the 599 strains of *E. coli*, an identity of biotype among cultures was seen in each of the following situations: (i) when many colonies picked from a pure culture of an identified strain were tested simultaneously; (ii) when a single culture stored on non-selective medium was tested on many different occasions over long periods—up to 4 years—of storage; (iii) when all isolates from sequential urine specimens from an individual patient were tested; (iv) when many colonies, picked from the same culture plate during the diagnostic examination of single urine specimens, were tested. In this series, there were only five strains, sequential isolates of which showed in-vivo variation in biotype characters. For three of the strains, minor variations to auxotrophy occurred (see above); for the other two, the changes were in the ornithine or lysine decarboxylase characters. Thus, biotypes were highly stable and reproducible, not only when many colonies were biotyped at the time of isolation of a strain, but also when cultures were retyped after storage.

Usefulness of biotyping; selected clinical cases

When biotyping methods were applied to the routine typing of isolates of *E. coli* isolated either from the urine of patients with long-term urinary-tract infection or from

TABLE IV

Distribution of 599 strains* of Escherichia coli among the observed primary biotypes and subtypes in the new biotyping scheme

Primary biotype no.	Number of strains	Subtypes† observed in the primary biotype (and number of strains in subtype)	Number of full biotypes
1	110	a(16), b(1), bd(1), bde(4), bdy(1), be(1), cd(2), cdef(2), d(27), de(29), def(1), defz(1), dey(1), df(2), dy(1), e(11), ef(1), efx(1), ex(2), ey(1), ez(2), x(1), z(1)	23
2	42	a(3), bd(3), bde(3), bdf(1), bf(1), d(8), de(8), def(1), dex(1), dx(2), e(10), x(1)	12
3	49	a(6), b(2), bcde(1), bde(3), be(1), cd(2), cde(4), ce(1), d(14), de(7), dex(1), e(4), ef(1), z(2)	14
4	12	a(1), bde(1), cde(1), cdefx(1), d(1), de(5), df(1), e(1)	8
5	49	a(11), bd(1), cdy(1), ce(1), d(21), de(5), dez(1), e(5), f(1), z(2)	10
6	21	a(2), bde(2), bdef(1), cd(1), cde(1), d(5), de(2), def(1), df(1), e(3), x(1), z(1)	12
7	19	bcd(1), bcdf(1), bde(3), bdz(1), be(1), bez(1), cdx(1), d(2), def(3), dz(1), e(1), ef(1), f(2)	13
8	11	a(1), bcde(1), bde(1), bz(1), c(1), cd(1), def(1), dz(1), ef(1), f(2)	10
9	81	a(25), b(1), bdef(1), bez(2), d(15), de(3), def(1), defy(1), dex(1), dey(4), dez(1), df(1), dfy(1), dx(2), dz(3), e(10), ex(2), f(1), x(5), z(1)	20
10	9	a(2), d(2), df(1), dy(1), e(1), ex(1), x(1)	7
11	10	a(1), d(4), de(1), def(1), df(2), e(1)	6
12	13	a(1), bef(1), bez(1), cde(2), cef(1), d(2), de(1), def(2), e(2)	9
13	21	a(3), bdf(1), ce(1), cf(2), d(4), de(1), def(1), defz(1), dx(1), e(2), f(2), fz(1), x(1)	13
14	18	a(1), b(1), bf(1), cdefx(1), cdy(1), d(3), de(2), def(2), df(1), ef(4), f(1)	11
15	65	a(12), b(2), bcd(1), bcde(1), bcdef(1), bcf(1), bd(1), bde(3), be(1), bf(4), cd(5), cde(3), cf(1), cfz(1), d(5), de(8), def(4), e(5), ef(2), f(4)	20
16	69	a(15), b(4), bcdef(1), bd(4), bdef(1), bdf(1), be(1), bef(1), bf(1), c(1), cdf(3), ce(5), cef(1), cez(1), cz(1), d(2), de(2), def(2), dey(1), df(3), e(9), ef(4), ez(1), f(3), z(1)	25
Any	599		213

* See footnote*, table I.

† See table III.

other sites, the results indicated relationships among sequential isolates from individual patients and showed when different strains were present (table V). For example, 13 catheter specimens of urine from patient F1 over a 15-month period yielded 13 isolates of *E. coli*. The finding that 12 of them (days 10–454) were of a biotype different in four characters from that of the first isolate (day 1) showed the presence of two distinct strains. Again, during 439 days, patient M1 yielded 12 urinary isolates of *E. coli*, 10 of biotype 5f and two of biotype 1bde; the latter (days

TABLE V
Biotypes of 40 isolates of Escherichia coli from six patients

Patient	Number of isolates	Site/days of isolation	Biotype characters*										Full biotype†
			raf	sor	orn	dul	rha	lys	aes	mot	fim	pro	
F1	13	Urine/1	+	+	-	+	-	+	+	+	+	+	3b
		Urine/10, 19, 22, 29, 43, 134, 171, 221, 241, 275, 401, 454	+	+	+	-	+	+	-	+	+	+	2d
M1	12	Urine/1, 19, 144, 151, 218	+	-	+	+	+	+	+	+	-	+	5f
		Urine/293, 294	+	+	+	+	-	+	-	-	+	+	1bde
		Urine/313, 355, 376, 399, 439	+	-	+	+	+	+	+	+	-	+	5f
F2	6	Urine/1, 4	+	+	-	+	+	+	-	+	+	+	3d
		Urine/42	-	+	-	+	+	+	-	-	-	+	11def
		Urine/168	+	-	-	+	-	+	+	-	+	+	7be
		Urine/233	+	-	-	+	+	+	-	-	-	+	7def
		Urine/246	-	-	-	-	-	+	-	-	-	+	16def
F3	3	Blood, sputum, urine/1	-	+	+	+	+	+	-	-	+	-	9dex
F4	4	Urine/1, 4, 9, 16	+	+	+	+	+	+	-	-	+	+	1de
N1	2	Blood, spinal fluid/1	+	+	+	+	+	+	-	-	+	+	1de

+ = Positive, - = negative result.

* For explanation of biotype-test characters, see table I.

† For biotype designations, see tables II and III.

293-294) were isolated when the patients had diarrhoea and might have been faecal contaminants. The discriminating potential of biotyping was seen with the urinary isolates from patient F2 from whom five different strains of five different biotypes were isolated during 8 months. In contrast, the isolation of *E. coli* of identical biotype from the blood, urine and sputum of patient F3 indicated the presence of a single strain at these sites. Finally, the urine of patient F4 before and after the birth of her child (N1) yielded isolates of a strain of *E. coli* of biotype 1de identical with those isolated from the blood and cerebrospinal fluid of N1 on the day of birth (day 7 of the mother's case history). That finding indicated the possible source of the single infecting strain associated with N1.

Other examples of the helpfulness of biotyping in clinical practice have been described (Crichton and Old, 1980; Old *et al.*, 1980; Wilson *et al.*, 1981).

DISCUSSION

In any typing system for the discrimination of members of a bacterial species, the following elements are essential: the ability to type a large proportion of the isolates, a high discriminating ability, reliability and reproducibility, and ease of performance for routine purposes. Thus, of the 22 tests originally examined by Crichton and Old (1979) for their ability to discriminate among strains of *E. coli*, 12 were discarded because they gave results that were: unreliable (salicin and aesculin hydrolysis in liquid

medium); too difficult to interpret (decarboxylation of arginine); poorly discriminatory (i.e., $\leq 6\%$ of strains of the minority type recognised) and so did not justify their routine inclusion in the biotyping scheme (fermentation of adonitol, L-arabinose, D-cellobiose, *m*-inositol, lactose, maltose, D-sorbitol, D-trehalose and D-xylose).

In the proposed biotyping scheme, the tests chosen for primary use differentiated almost equal numbers of positive and negative types among the 599 strains in this series, and the results in these tests, with raffinose, sorbose, ornithine and dulcitol, were reproducible on different occasions of testing the same strain. Furthermore, all strains were typable by this scheme and examples of each of the 16 theoretically attainable primary biotypes were detected.

Tests for use at the secondary level included some that differentiated only 10–15% of strains (rhamnose fermentation, lysine decarboxylation and prototrophy) and others that required considerable expertise for correct interpretation of the results (aesculin hydrolysis on agar, motility and type-1 fimbriation). Nevertheless, the secondary tests subdivided strains of a single biotype into many full biotypes and so provided greater strain discrimination.

Among lactose-fermenting strains, a strong correlation existed between fermentation of sucrose and raffinose (Crichton and Old, 1979), although strains of the rare sucrose-negative and raffinose-positive phenotype were encountered on occasion (Old *et al.*, 1980). Although some additional discrimination of sucrose-fermenting strains may be obtained by reference to the sucrose-weak and sucrose-strong phenotypes (Crichton and Old, 1980; Old *et al.*, 1980; Wilson *et al.*, 1981), this latter discrimination of types could not readily be incorporated into the biotyping scheme even at the secondary level. Accordingly, because, for the majority of strains of *E. coli*, the use of both raffinose and sucrose generally yielded little more information than was obtained from the use of either alone the sucrose test, which was less reliable to perform, was excluded from the present system, although other schemes have included both (Buckwold *et al.*, 1979).

In the establishment of a biotyping scheme for the discrimination of members of a bacterial species, it is important to distinguish tests that are more appropriate for identification of the species than for discrimination of types within the species. For *E. coli*, tests in the former category would be, for example, indole production (99% positive), adonitol fermentation (96% negative) or urea hydrolysis ($> 99\%$ negative), because most strains are clearly positive or negative with regard to these characters (Edwards and Ewing, 1972; Wachsmuth, Davis and Allen, 1979). Though primarily species-identifying tests, these are also extremely valuable for the discrimination of rare strains that give the result atypical for the species, i.e., indole-negative, adonitol-fermenting, or ureolytic. However, the rare occurrence of atypical strains with aberrant properties does not justify the general use of these tests in the biotyping of all isolates of *E. coli*. Discriminating tests, on the other hand, are those that separate strains optimally into positive and negative types at the definitive time of reading of the test, and the best discriminating tests are those that do not depart too far from the optimum (50:50). Despite that difference in purpose, identifying and discriminatory tests are often used together in biotyping schemes. Although the usefulness of some, but not all, of the substrates selected by us for biotype discrimination of *E. coli* has been recorded by others (see Mushin and Ashburner, 1964; Bettelheim *et al.*, 1974; Sakazaki, Tamura and Nakamura, 1974; van der Waaij *et*

al., 1975; Myerowitz *et al.*, 1977; Scotland, Gross and Rowe, 1977; Magalhães and Vance, 1978; Buckwold *et al.*, 1979), different workers have recommended different ones of them for discrimination. Some have been included, however, in biotyping schemes along with tests that afford information more appropriate to the identification of species than the discrimination of types.

In an important study of urinary strains, which were discriminated by biotyping independently of serotyping (Davies, 1977), 55 biotypes were detected among 574 strains of *E. coli* biotyped with the API 20E Enterobacteriaceae kit (API Laboratory Products, Rayleigh, Essex). However, because that system is primarily one of identification and because 16 of its 21 tests provide little or no discrimination for biotypes of *E. coli*, it was not surprising that six biotypes accounted for almost 70% of the strains. An improved scheme of nine tests (Buckwold *et al.*, 1979) included five (rhamnose, lysine, raffinose, sucrose and motility) independently recommended by Crichton and Old (1979) and four others (melibiose, adonitol, lactose and β -haemolysis), not favoured by us because of poor discrimination, duplication of information or possible plasmid control. The scheme of Buckwold *et al.* (1979) separated 959 strains into 78 biotypes, 11 of which accounted for 67% of the strains. The discrimination achieved by the present two-tier system recognised 213 full biotypes among 599 strains, 10 of which accounted for 31% of our strains. The flexibility of the two-tier system is great, and other tests that may be shown in the future to be useful for strain discrimination can readily be accommodated as secondary tests within the system. There is also similar provision even for the inclusion of tests, e.g., adonitol, lactose, that achieve lower discrimination than those we have recommended.

The observations on biotype reproducibility *in vivo* and *in vitro* indicated that biotyping was stable enough for prospective and retrospective analyses. The unreliability of biotyping claimed by earlier workers (Bettelheim and Taylor, 1969) is difficult to understand but may have resulted from inattention to the need for clearly defined times of reading for each test.

Even with a system such as the present biotyping scheme, which provides such accurate and reliable typing information, the assumption should not be made that isolates similar by a single typing system are of clonal origin. Thus, isolates from widely different sources may be wrongly classed as similar if they belong to a common type and, conversely, isolates of the same strain may be wrongly classed as different if the strain in the course of its spread has undergone *in-vivo* variation in one or more typing characters. Information from any one typing scheme, therefore, should ideally be supplemented with data from additional methods. Accordingly, in our studies resistotyping, haemagglutinin typing, partial O serogrouping, colicine typing and antibiogram resistances have been used as suitable ancillary techniques with which to support biotyping (Crichton and Old, 1980; Old *et al.*, 1980; Wilson *et al.*, 1981). All strains are typable by biotyping and by resistotyping, which together are sufficient in most cases to define isolates as identical or non-identical. Minor variations in characters may arise during spread of a strain of *E. coli* and in these rare circumstances accurate strain identification can be obtained by the gradual extension of the biotype-resistotype profile with results from additional techniques.

REFERENCES

- BETTELHEIM, K. A. AND TAYLOR, J. 1969. A study of *Escherichia coli* isolated from chronic urinary infection. *J. med. Microbiol.*, **2**, 225.

- BETTELHEIM, K. A., TEOH-CHAN, C.H., CHANDLER, M. E., O'FARRELL, S. M., RAHAMIN, L., SHAW, E. J. AND SHOOTER, R. A. 1974. Further studies of *Escherichia coli* in babies after normal delivery. *J. Hyg., Camb.*, **73**, 277.
- BUCKWOLD, F. J., RONALD, A. R., HARDING, G. K. M., MARRIE, T. J., FOX, L. AND CATES, C. 1979. Biotyping of *Escherichia coli* by a simple multiple-inoculation agar plate technique. *J. clin. Microbiol.*, **10**, 275.
- COWAN, S. T. 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed., Cambridge University Press, Cambridge, p. 148.
- CRICHTON, P. B. AND OLD, D. C. 1979. Biotyping of *Escherichia coli*. *J. med. Microbiol.*, **12**, 473.
- CRICHTON, P. B. AND OLD, D. C. 1980. Differentiation of strains of *Escherichia coli*: multiple typing approach. *J. clin. Microbiol.*, **11**, 635.
- DAVIES, B. I. 1977. Biochemical typing of urinary *Escherichia coli* strains by means of the API 20E Enterobacteriaceae system. *J. med. Microbiol.*, **10**, 293.
- DUGUID, J. P., ANDERSON, E. S., ALFREDSSON, G. A., BARKER, R. AND OLD, D. C. 1975. A new biotyping scheme for *Salmonella typhimurium* and its phylogenetic significance. *J. med. Microbiol.*, **8**, 149.
- DUGUID, J. P., SMITH, I. W., DEMPSTER, G. AND EDMUNDS, P. N. 1955. Non-flagellar filamentous appendages ('fimbriae') and haemagglutinating activity in *Bacterium coli*. *J. Path. Bact.*, **70**, 335.
- EDWARDS, P. R. AND EWING, W. H. 1972. Identification of Enterobacteriaceae, 3rd ed., Burgess Publishing Co., Minneapolis.
- MACCACHARO, G. A. AND HAYES, W. 1961. The genetics of fimbriation in *Escherichia coli*. *Genet. Res., Camb.*, **2**, 394.
- MAGALHÃES, M. AND VANCE, M. 1978. Hydrogen sulphide-positive strains of *Escherichia coli* from swine. *J. med. Microbiol.*, **11**, 211.
- MEYNELL, G. G. AND MEYNELL, E. 1965. Theory and practice in experimental bacteriology, Cambridge University Press, Cambridge, p. 35.
- MUSHIN, R. AND ASHBURNER, F. M. 1964. Ecology and epidemiology of coliform infections: II. The biochemical reactions and drug sensitivity of coliform organisms. *Med. J. Aust.*, **1**, 303.
- MYEROWITZ, R. L., ALBERS, A. C., YEE, R. B. AND ØRSKOV, F. 1977. Relationship of K1 antigen to biotype in clinical isolates of *Escherichia coli*. *J. clin. Microbiol.*, **6**, 124.
- OLD, D. C., CRICHTON, P. B., MAUNDER, A. J. AND WILSON, M. I. 1980. Discrimination of urinary strains of *Escherichia coli* by five typing methods. *J. med. Microbiol.*, **13**, 437.
- SAKAZAKI, R., TAMURA, K. AND NAKAMURA, A. 1974. Further studies on enteropathogenic *Escherichia coli* associated with diarrhoeal diseases in children and adults. *Jap. J. med. Sci. Biol.*, **27**, 7.
- SCOTLAND, S. M., GROSS, R. J. AND ROWE, B. 1977. Serotype-related enterotoxigenicity in *Escherichia coli* O6.H16 and O148.H28. *J. Hyg., Camb.*, **79**, 395.
- VAN DER WAAIJ, D., SPELTIE, T. M., GUINÉE, P. A. M. AND AGTERBERG, C. 1975. Serotyping and biotyping of 160 *Escherichia coli* strains: comparative study. *J. clin. Microbiol.*, **1**, 237.
- WACHSMUTH, I. K., DAVIS, B. R. AND ALLEN, S. D. 1979. Ureolytic *Escherichia coli* of human origin: serological, epidemiological, and genetic analysis. *J. clin. Microbiol.*, **10**, 897.
- WILSON, M. I., CRICHTON, P. B. AND OLD, D. C. 1981. Characterisation of urinary isolates of *Escherichia coli* by multiple typing: a retrospective analysis. *J. clin. Path.*, **34**, 424.