BIOCHEMICAL TYPING OF URINARY *ESCHERICHIA COLI* STRAINS BY MEANS OF THE API 20 E ENTEROBACTERIACEAE SYSTEM

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IN the management of patients with recurrent urinary infection it is essential to discover whether failure of treatment is due to recrudescence of infection with the original causative organism or whether there has been reinfection with a different organism. High dosage chemotherapy may be required to treat recrudescence (Brumfitt and Reeves, 1969) but reinfection may need completely different management and, possibly, the use of continuous long-term prophylactic antimicrobial therapy. When different species of bacteria cause second or subsequent episodes of infection there is no doubt that reinfection is the problem but, when all episodes are caused by *Escherichia coli*, further identification, such as serological or biochemical typing, is necessary to define the correct management. In domiciliary practice about 85% of uncomplicated urinary infections are caused by *E. coli* (Davies, Mummery and Brumfitt, 1975), and the same *E. coli* serotype has been found in the urine of some patients for up to 30 weeks despite several courses of chemotherapy with conventional doses (Ganguli, 1970).

Most bacteriological laboratories are unable to carry out extensive serological studies on isolates of *E. coli* as they must rely on the available range of commercial antisera or prepare their own, the latter course requiring an animal house and much time for immunisation of the animals and purification of the sera. Because of the difficulties inherent in serological typing, an attempt has been made to use for typing a numerical biotype code system based on the results of 21 standardised biochemical tests (the API 20 E Enterobacteriaceae System, A.P.I. Laboratory Products, Rayleigh, Essex) presented in a readily available kit form.

MATERIALS AND METHODS

Mid-stream specimens of urine were collected after simple perineal washing with distilled water from patients attending a special urinary infection clinic at Edgware General Hospital and were cultured by a semi-quantitative method (Leigh and Williams, 1964). Organisms present in significant numbers (10^5 or more per ml) were identified by standard methods (Cowan and Steel, 1974) and sensitivity tests carried out with "Multodisks" (Oxoid Ltd).

Each strain was inoculated into a set of API 20 E tests in accordance with the manufacturer's instructions with all oxidase-negative Gram-negative bacilli suspended in sterile water. The 20 basic tests were augmented by adding fresh oxidase reagent (1% aqueous tetramethyl-p-phenylenediamine solution) to one of the tubes that had not shown a colour change (usually the negative hydrogen sulphide production tube). After overnight incubation, appropriate standard reagents were added and the results noted. The 21 tests in the

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TABLE I
Biochemical test results for strains of Escherichia coli

Test	Percentage number of strains positive	Commonest code numbers
ONPG	99.5	} 1, 5
Arginine dehydrolase	2.8	
Lysine decarboxylase	69.0	
Ornithine decarboxylase	71.3	} 1, 0
Citrate utilisation	...	
H ₂ S production	...	
Urease production	...	} 4
Tryptophane deaminase	...	
Indole production	100.0	
Voges-Proskauer	...	} 4
Gelatin liquefaction	...	
Fermentation of glucose	100.0	

TABLE II
Carbohydrates fermented by 574 strains of Escherichia coli

Carbohydrate	Percentage number of strains positive	Commonest code numbers
Mannitol	99.5	} 5
Inositol	0.2	
Sorbitol	94.9	
Rhamnose	89.0	} 1, 5, 7
Saccharose	40.6	
Melibiose	85.5	
Amygdalin	0.7	} 2
Arabinose	98.8	
(Oxidase)	...	

series were then divided into seven groups of three, and points allotted for positive results, one point being given for the first test in each group of three, two for the second, and four for the third. Seven numbers, each varying from 0 to 7, were thus produced. These numbers formed the numerical code for each of the 574 isolates of *E. coli* examined. Additionally, 140 *E. coli* strains were typed serologically, with antisera made and absorbed in my own laboratory.

RESULTS

The results of the biochemical studies on 574 *E. coli* strains are shown in tables I and II. In view of the lack of differentiation provided by some of the tests (e.g., indole production, fermentation of glucose, mannitol and sorbitol) it might have been expected that biotyping would not distinguish many different types but this proved not to be the case. Tests for lysine and ornithine

TABLE III
Biotype frequencies among 574 strains of Escherichia coli

Biotype code	Number of strains	Percentage of total
5, 144, 552	133	23.2
5, 144, 572	109	19.0
5, 044, 552	48	8.4
1, 144, 572	40	6.9
1, 144, 552	34	6.0
1, 044, 552	30	5.2
5, 144, 512	28	4.9
1, 044, 562	21	3.6
5, 144, 532	11	1.9
46 different biotypes	1-9 of each	20.9

TABLE IV
Pretreatment biotype and serotype results for 50 patients

Number of patients	Number and relationships of biotypes (codes) and serotypes obtained
41	98 similar codes and serotypes
2	4 different codes, different serotypes
7	7 pairs (14 cultures) with similar serotypes, different biotypes

decarboxylase production were very helpful in differentiation (69% and 71% of strains respectively showing such activities), and the fermentation of saccharose (41% of strains) was a further useful marker.

The frequency with which the commonest biotypes were found is shown in table III; it can be seen that two biotypes accounted for 42% of the strains tested. The remaining 332 strains were separated into 53 different biotypes of which each accounted for between 1.9 and 8.4% of the strains isolated. The remaining 46 biotypes were found less often.

To study the reproducibility of the API 20 E system, paired cultures taken from 50 patients before treatment were examined biochemically and serologically and any discrepancies recorded. The results are presented in table IV. Discrepancies were observed in only seven of the 50 pairs studied. In each of two other patients, differing *E. coli* serotypes were found in the two pre-treatment urine specimens, this being consistent with the differing biotype results.

When the serotypes of 140 *E. coli* strains were compared with their biotypes (table V) it was found that the commonest biotypes were spread over a considerable number of different O-serotypes, there being little correlation between the two typing systems.

TABLE V
Numbers of *Escherichia coli* serotypes in each biotype (140 strains tested)

Biotype code	Number of the stated biotype serotyped as										Number of isolates tested†
	NT*	O1	O2	O4	O5	O6	O7	O11	O18	O75	
5, 144, 552	13	3	...	10	...	6	...	1	7	2	42
5, 144, 572	11	7	6	1	...	...	1	...	...	10	36
5, 044, 552	13	1	...	...	...	1	...	...	...	...	15
5, 144, 512	7	4	...	2	...	...	...	...	...	...	13
1, 144, 572	...	2	1	...	...	...	...	...	...	2	5
5, 144, 532	...	...	...	2	...	...	...	...	...	...	2
5, 144, 172	3	...	...	...	...	...	...	...	...	...	3
5, 044, 562	...	...	...	...	1	...	2	...	...	...	3
7, 044, 562	...	...	...	...	...	2	...	...	...	...	2
6 further types	7	3	1	2	2	1	...	3	...	...	19

* NT = Not typable with 11 available *E. coli* antisera.

† Total number of isolates = 140.

DISCUSSION

Grüneberg, Leigh and Brumfitt (1968) summarised the results of serotyping urinary *E. coli* strains with a wide range of somatic antisera that are not generally available, and produced evidence of local as well as national variation in serotype prevalence. Other studies—for example Kunin, Deutscher and Paquin (1964), and Davies, Mummery and Brumfitt (1975)—have shown that many urinary infections are caused by a small number of different *E. coli* serotypes. The proportion caused by the 11 commonest serotypes varies from 55 to 70% (Rantz, 1962; McGeachie, 1965; Grüneberg and Bettelheim, 1969; Ganguli, 1970; Dootson, Maclaren and Titcombe, 1973), but some strains cannot be typed at all because they are auto-agglutinable or have lost antigens.

The biochemical properties of *E. coli* and other enterobacteria have been used in the development of the multi-tube API 20 E kit system. With the kit, together with an oxidase test, a biochemical code (a biotype) can be produced for every member of the Enterobacteriaceae and for many other intestinal Gram-negative bacilli (for example, oxidase-positive organisms such as *Pseudomonas aeruginosa*). This compares very well with the serotyping system, a method able to account for only 55–70% of *E. coli* strains with presently available antisera. There is the additional advantage that a profile register compiled by the manufacturers enables the biotype codes to be interpreted in any country in which the kit is used. Furthermore, this biochemical test system forms the basis for primary identification of the organisms under test (Willis and Cook, 1975), as it will distinguish, for example, between *E. coli*, *Klebsiella pneumoniae* and *Serratia marcescens* by the production of different biotype codes or numerical profiles which can be interpreted by reference to the API profile register.

As shown by Willis and Cook (1975) the results of these tests are reproducible. Results of the present studies on paired cultures taken from patients with urinary infection before treatment (table IV) show that in only seven of the 50

patients so investigated was there any discrepancy within the pairs. In these seven pairs of cultures both members of the pair were the same *E. coli* serotype, but the biotype codes differed. In one patient the two numerical codes were completely different, but in the other six there were variations only in the interpretation of the lysine decarboxylase test. (The manufacturers recognise the difficulty in assessing the results of this test and recommend that any change from yellow towards orange or orange-red within 24 h be recorded as a positive result).

In an extensive study of patients with chronic urinary infection, Bettelheim and Taylor (1969) were able to use a full range of antisera prepared against somatic O-, surface K- and flagellar H-antigens of *E. coli*. They also carried out biotyping by testing decarboxylase production and the fermentation of 16 different carbohydrate substrates. After finding that biotype results were not always reproducible, mainly as a result of difficulties with the decarboxylase tests, these workers found that, as in the present study (table V), there was little relationship between biotype and serotype results. As biochemical tests are easily performed and are commonly used to identify bacterial species (Cowan and Steel, 1974), it is difficult to agree with claims that biotyping is valueless in primary identification (Bettelheim and Taylor, 1969). Indeed, it is not readily apparent what other system could be used because serological typing is time-consuming (especially if full antigen analysis is to be performed in a reference laboratory, and is not concerned in primary identification).

Although it is possible to obtain a more exact description of an individual *E. coli* strain by combined serotyping and biotyping, this is necessary in special circumstances only, e.g., cross-infection within a ward or theatre unit. An attempt was made by Hettiaratchy, Cooke and Shooter (1973) to combine serotyping and colicine typing of *E. coli* strains but less than one third of the organisms tested formed demonstrable colicines and this reveals the limitations of their approach.

The decision to admit a patient to hospital for high-dosage antibacterial therapy or to use long-term antibiotic treatment is of great importance. The bacteriological laboratory can help in reaching a balanced decision, by providing information that distinguishes between relapsing urinary infections (with similar biotype or serotype results) and reinfections, when different *E. coli* types are shown to be responsible. Each bacteriology laboratory that supports a urinary infection clinic must decide on a suitable typing method if it is to encourage the development of rational chemotherapy.

SUMMARY

With the API 20 E Enterobacteriaceae system of biochemical testing, a biotype, coded numerically, was determined for each of 574 strains of *Escherichia coli* isolated from patients with urinary tract infection. The serotypes of the strains were also determined.

Fifty-five different biotypes were identified, two accounting together for 42% of the strains examined and seven others each accounting for between 8.4 and 1.9%. There was little correlation between biotype and serotype.

Fifty pairs of strains were isolated from patients before treatment. In 43 the biotype and serotype of both strains of each pair were the same. In six pairs the biotypes, but not the serotypes, differed, the difference being limited to the results of the tests for lysine decarboxylase. The biotypes of the strains of the remaining pair differed widely although their serotypes were the same.

It is suggested that this method of biotyping offers a simple but accurate way of discriminating between recrudescing urinary tract infection caused by *E. coli* and that due to reinfection.

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REFERENCES

- BETTELHEIM, K. A. AND TAYLOR, J. 1969. A study of *Escherichia coli* isolated from chronic urinary infection. *J. med. Microbiol.*, **2**, 225.
- BRUMFITT, W. AND REEVES, D. S. 1969. Recent developments in the treatment of urinary tract infections. *J. infect. Dis.*, **120**, 61.
- COWAN, S. T. AND STEEL, K. J. 1974. Manual for the identification of medical bacteria, 2nd ed., London.
- DAVIES, B. I., MUMMERY, R. V. AND BRUMFITT, W. 1975. Ampicillin, carbenicillin indanyl ester, and nifuratel in the treatment of urinary infection in domiciliary practice. *Br. J. Urol.*, **47**, 335.
- DOOTSON, P. H., MACLAREN, D. M. AND TITCOMBE, D. H. M. 1973. The distribution of urinary O-groups of *Escherichia coli* in urinary infections and in the normal faecal flora. In *Urinary tract infection*, edited by W. Brumfitt and A. W. Asscher, London, p. 139.
- GANGULI, L. 1970. Serological grouping of *Escherichia coli* in bacteriuria in pregnancy. *J. med. Microbiol.*, **3**, 201.
- GRÜNEBERG, R. N. AND BETTELHEIM, K. A. 1969. Geographical variation in serological types of urinary *Escherichia coli*. *J. med. Microbiol.*, **2**, 219.
- GRÜNEBERG, R. N., LEIGH, D. A. AND BRUMFITT, W. 1968. *Escherichia coli* serotypes in urinary tract infection: studies in domiciliary, ante-natal and hospital practice. In *Urinary tract infection*, edited by F. O'Grady and W. Brumfitt, London, p. 68.
- HETTIARATCHY, I. G. T., COOKE, E. M. AND SHOOTER, R. A. 1973. Colicine production as an epidemiological marker of *Escherichia coli*. *J. med. Microbiol.*, **6**, 1.
- KUNIN, C. M., DEUTSCHER, R. AND PAQUIN, A. 1964. Urinary tract infections in school children: an epidemiologic, clinical, and laboratory study. *Medicine, Baltimore*, **43**, 91.
- LEIGH, D. A. AND WILLIAMS, J. D. 1964. Method for the detection of significant bacteriuria in large groups of patients. *J. clin. Path.*, **17**, 489.
- MCGEACHIE, J. 1965. Serological grouping of urinary *Escherichia coli*. *J. clin. Path.*, **18**, 428.
- RANTZ, L. A. 1962. Serological grouping of *Escherichia coli*. Study in urinary tract infection. *Archs intern. Med.*, **109**, 37.
- WILLIS, G. AND COOK, I. J. Y. 1975. Enterobacteriaceae identification: a comparative study of API, Encise and conventional methods. *The med. Technologist*, **5**, No. 1, 4.