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COLICINE PRODUCTION AS AN EPIDEMIOLOGICAL MARKER OF *ESCHERICHIA COLI*

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THE epidemiology of *Escherichia coli* is poorly understood, and although serological characterisation of strains affords an excellent method of typing *E. coli*, it is time-consuming and can be performed only in specialised laboratories. Another method would be of value. Colicine typing as a method of differentiating strains has been used (Linton, 1960; McGeachie, 1965; Mustafa and Dunbar, 1970), but not so extensively as serotyping and its usefulness has not been defined.

Colicine typing is simple to perform and has been found valuable in epidemiological studies on shigellae (Abbott and Shannon, 1958; Gillies, 1964; Hart, 1965), particularly on strains of *Shigella sonnei* because they are serologically and often biochemically homogeneous (Hart).

This paper records our experience of colicine typing of *E. coli* and of the validity of the method as an epidemiological tool. Serological and colicine typing were used in this investigation to determine whether the combination of the two methods would provide a finer subdivision of strains, to show the frequency of colicine production and sensitivity among serologically defined strains, and to show any correlation between colicine types and antigenic structure of the organisms. This is particularly interesting because several workers attempting to purify colicines have observed a close association between colicines and the O antigen of the organism (Goebel and Barry, 1958; Hutton and Goebel, 1961; Barry, Everhart and Graham, 1963).

An epidemiological application of the typing method is also reported.

MATERIALS AND METHODS

Specimens of faeces were collected from patients in a female, open medical ward as soon as possible after admission and thereafter at weekly intervals for 1 yr. The patients were those usually seen in a general medical ward and particularly included diabetics.

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Isolation of E. coli from the faeces and its identification were performed as described previously (Cooke, Ewins and Shooter, 1969). Five colonies of each colonial type were examined from each specimen. Serological identification using antisera to O groups 1-25, 39, and 75 was made as before (Cooke *et al.*, 1969).

Determination of colicine production. In this investigation, two methods of detecting colicine production and two methods of colicine typing were used. The first 300 strains were screened for colicine production by Abbott and Shannon's plate-diffusion technique and by McGeachie's colicine-extraction method. Later isolates were screened by plate-diffusion only. *E. coli* strains Row and phi were used as indicators in the preliminary screening.

In Abbott and Shannon's method the test strain was grown on tryptone soya blood agar. After incubation for 48 hr at 37°C the growth was killed by exposure to chloroform vapour and removed by scraping with a glass slide. To each plate, 5-hr broth cultures of each of the indicator strains were then applied as streaks at right-angles to the line of the original inoculum, and the zones of inhibition were noted after overnight incubation at 37°C.

In McGeachie's method the test strain was grown on digest broth agar. After incubation for 24 hr it was killed by exposure to chloroform vapour, then frozen at -15°C overnight, and thawed the next morning. The expressed supernatant fluid was spot-inoculated on a lawn of indicator strain spread on peptone-water agar. The cultures were examined for zones of inhibition after overnight incubation at 37°C.

All colicine-producing strains were next typed in parallel by Abbott and Shannon's method and by Gillies' modification of it. The main difference between these methods is that the period of pre-diffusion is 48 hr in Abbott and Shannon's method and 24 hr in Gillies' technique. Ten indicators of colicinogenicity were used: seven *Sh. sonnei* (strains 2/7, 56, 17, 56/56, 2, R6, R5), one *Sh. schmitzi* (strain M19), and two *E. coli* (strains Row and phi). The shigellae were those used by Abbott and Shannon and by Gillies for typing shigellae and by McGeachie for typing *E. coli*.

Colicine sensitivity testing. The sensitivities of the strains to colicines V, A, B, K, H, E1, E2, and I were determined by the method of Abbott and Shannon in tests with standard producer strains. *E. coli* (phi) was streaked on every plate to ensure that the producer strain did produce colicine under the test conditions. All tests were done in duplicate.

RESULTS

From 1243 specimens of faeces, 1401 strains of *E. coli* were isolated; from 93 of the specimens *E. coli* was not isolated. Of the 1401 isolates of *E. coli*, 965 (49.6 per cent.) could be serologically characterised, 586 (41.8 per cent.) strains were smooth but not agglutinated by any of the antisera used, and 120 (8.6 per cent.) strains were rough.

Of the 695 serologically defined strains, 243 (35 per cent.) produced colicines and were contained in 19 type patterns (table I). The type designation was devised for this study because no colicine-typing scheme for *E. coli* has previously been fully described.

Comparison of methods

Abbott and Shannon's plate-diffusion technique for detection of colicine production and McGeachie's colicine extraction method were found to be equally sensitive. For 279 (93 per cent.) of the 300 strains tested the two methods gave identical results. Eleven strains (3.7 per cent.) were shown to be colicine producers by Abbott and Shannon's technique, whilst extracts of these strains prepared by McGeachie's method did not yield detectable colicine. On the other hand, 10 strains (3.3 per cent.) produced detectable amounts of

colicines by McGeachie's extraction method but not by Abbott and Shannon's technique.

For colicine typing, the two methods gave identical results in most instances. Seven of 249 (2.9 per cent.) strains gave different patterns of inhibition by the two methods. In five of the seven instances, inhibition of some of the indicators was observed only when strains were typed by Gillies' method whilst in the other two instances inhibition of some of the indicators was observed only when strains were typed by Abbott and Shannon's method.

TABLE I
Patterns of inhibition of the indicator strains that characterised the colicine types of Escherichia coli

Colicine type	Inhibition of indicator strain number								
	56	17	56/56	2	R6	M19	2/7	R5	Row phi
1	+	+	+	+	-	+	-	-	+
2	-	-	-	-	-	-	-	-	+
3	-	-	-	-	-	-	-	-	+
4	+	+	-	+	+	-	+	-	+
5	-	+	-	+	-	-	-	-	+
6	+	-	-	-	+	-	-	-	+
7	+	+	+	+	-	-	+	+	+
8	+	+	+	+	+	-	+	+	+
9	+	+	-	+	+	+	+	-	+
10	+	+	-	-	+	-	-	-	+
11	+	-	+	+	+	-	-	-	+
12	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	-
14	+	+	+	+	+	-	-	-	+
15	+	+	+	+	+	+	+	-	+
16	+	-	+	-	+	-	-	-	+
17	+	+	+	-	+	-	-	-	+
18	+	+	+	+	+	+	-	-	+
19	-	-	-	-	-	+	-	-	+

+ = Inhibition. - = No inhibition.

Reproducibility of the typing technique

The validity of colicine typing as an epidemiological marker was assessed for both methods in three ways: first, by noting the constancy of types isolated from individuals in serial cultures; second, by determining the pattern of inhibition in multiple picks of the same serotype from single specimens; and third, by noting the stability of type on testing strains after storage on agar slopes at room temperature for 3, 6, 9, and 12 mth.

Constancy of type in replicate isolates. From 42 patients, isolates of the same serotype were obtained on two or more occasions. From 37 patients the same serotype and colicine type was isolated on each occasion from 2 to 13 cultures. In the remaining five patients, although the serotype remained constant in serial isolates, the colicine type varied (table II).

Of the 37 patients who yielded a constant serotype and colicine type, 18

excreted the same type for one or more weeks (table III), whilst in two patients the same type was still present even on re-admission to hospital.

TABLE II
Constancy of colicine type in replicate isolates of E. coli obtained on different occasions from the same patient: results from 42 patients

Number of replicate isolates of the same serotype from a patient*	Number of patients whose isolates were	
	all of one colicine type	of more than one colicine type
1	18	4
2	9	1
3	4	0
4	2	0
5 or more	4	0
Total	37	5

* Excluding the first strain isolated from the patient.

Reproducibility of type in single specimens. The second index of reliability was the uniformity of colicine type in 5–10 colonies of the same serotype from single specimens. Of the 23 specimens examined, 20 showed complete agreement in all colonies, two showed agreement in four out of five colonies and in one specimen only three of the five colonies agreed. Of the 196 colonies examined, 192 agreed (98.6 per cent.) The variation observed in the remaining four colonies was a loss of inhibition of only one indicator.

TABLE III
Serial isolations of E. coli of the same serotype and colicine type from 37 patients, in relation to the period over which they were isolated

Time in weeks during which replicate isolates of the same serotype and colicine type were obtained	Number of patients from whom such isolates were recovered
< 1	19
1	4
2	3
3	5
4	3
6	2
8	1

Effects of storage. Of 20 strains tested after various periods of storage, 18 showed a constancy of type. Two strains showed a loss of inhibition of one indicator when tested after 6 months' storage. This variation persisted when these strains were tested after further storage.

TABLE IV
Correlation between colicine typing and serological typing

Colicine type	Number of strains of indicated serotype																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23	24	25	39	75	All	
1	1	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	7	
2	2	6	4	1	1	0	4	1	0	0	0	0	0	0	2	0	3	0	1	0	0	0	0	0	0	0	25	
3	0	1	0	2	1	1	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	10	
4	1	5	0	1	0	2	3	0	0	0	2	0	1	0	1	0	2	0	2	4	1	1	0	1	1	1	29	
5	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	1	8	
6	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
7	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	
8	2	2	1	1	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	14	
9	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
10	0	5	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	10	
11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	2	
12	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	
13	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
16	1	4	0	4	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	
17	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	
18	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
19	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	
UT	14	15	15	28	5	14	10	17	3	2	20	7	4	2	22	1	31	5	6	13	4	6	0	20	8	21	293	
Total	28	42	20	39	11	21	26	22	3	2	22	10	6	3	28	1	37	8	9	20	6	8	0	22	11	24	429	

UT = Untypable strains (not producing colicine detectable with the indicator strains *E. coli* (Row) and *E. coli* (phi)).

Colicine production and sensitivity and its relation to serotype

Table IV shows the results of typing *E. coli* by serological and by colicine-production methods. There appears to be no relationship between colicine

TABLE V

Sensitivity to eight standard colicines among strains of different serotypes of E. coli

Serotype	Number of strains of the stated serotype									
	examined	that produced colicine	that were sensitive to colicine							
			V	A	B	K	H	E2	E1	1
1	10	4	0	1	5	5	2	6	1	1
2	10	8	0	0	1	7	0	1	0	0
3	10	1	0	0	3	9	2	6	2	1
4	10	2	0	0	0	10	1	1	1	1
5	10	6	0	0	0	7	1	2	2	3
6	10	3	0	0	6	10	1	3	1	0
7	10	9	0	0	4	7	0	8	0	0
8	9	4	0	1	1	6	1	9	7	4
9	3	0	0	0	0	2	0	2	1	0
10	3	1	0	0	0	2	0	1	1	0
11	10	0	0	3	1	8	3	2	1	1
12	8	3	0	0	0	5	2	2	0	0
13	6	1	0	0	0	5	0	2	2	1
14	3	1	0	1	2	3	0	1	1	2
15	10	0	0	1	3	7	5	0	1	1
17	1	0	0	0	1	1	0	0	0	0
18	10	1	0	0	0	10	3	0	0	1
19	8	3	0	0	5	7	4	4	2	1
20	10	4	0	3	8	10	0	3	2	1
21	10	3	0	0	4	9	1	2	0	0
22	6	2	0	0	0	6	1	2	0	0
23	8	2	0	0	0	7	0	0	0	0
25	9	1	0	0	1	8	2	5	2	2
39	10	3	0	1	0	8	2	2	0	0
75	10	2	0	0	0	9	0	3	1	0
Any	204	64	0	11	45	168	31	67	28	20
										17

type and serotype. Most serotypes could be subdivided into several colicine types and most colicine types contained members of two or more serotypes as did the untypable strains.

Similarly the activity of individual colicines was not restricted to certain serotypes. The colicine sensitivity of 204 strains is shown in table V. Of 204 strains, 17 (8.3 per cent.) were resistant to all the eight standard colicines against which they were tested. These resistant strains were scattered in 13 of 25 different serogroups. Of the 187 sensitive strains, 80 (39.2 per cent.) were inhibited by one colicine, 69 (33.8 per cent.) by two colicines and 38 (18.9 per cent.) by three or more colicines. Colicine K showed the greatest range of activity and inhibited 168 strains, whilst colicine V showed no activity at all.

Illustrative epidemiological application of the method

This part of the study was made to determine whether colicine typing of *E. coli* from the faeces of hospital patients was of value in indicating the sources of the strains. *E. coli* was isolated from single faecal specimens from normal people who did not work in the hospital and from weekly faecal specimens from patients in a female medical ward. All strains of defined serotype isolated

TABLE VI
Frequency distribution of colicine types of E. coli isolated from faeces of patients in a general medical ward

Colicine type	Number (and percentage) that belonged to the stated colicine type among	
	strains of defined serotype isolated during 12 mth	all strains isolated during 3 mth
4	29 (21.3)	12 (23.5)
2	25 (18.4)	9 (17.7)
8	14 (10.3)	6 (11.8)
16	12 (8.8)	5 (9.8)
3	10 (7.4)	3 (5.9)
10	10 (7.4)	4 (7.8)
5	8 (5.9)	0 —
1	7 (5.1)	3 (5.9)
17	6 (4.4)	1 (2.0)
12	3 (2.2)	1 (2.0)
6	2 (1.5)	1 (2.0)
11	2 (1.5)	0 —
19	2 (1.5)	3 (7.8)
7	1 (0.7)	0 —
9	1 (0.7)	0 —
13	1 (0.7)	0 —
14	1 (0.7)	2 (3.9)
15	1 (0.7)	0 —
18	1 (0.7)	1 (2.0)

from the patients during a year were colicine typed by the method of Abbot and Shannon and for a period of 3 mth all strains were colicine typed. *E. coli* was also isolated from food as it was being served to the patients in the ward, from meat on arrival at the hospital, and from articles used in the preparation of food and from the food itself in the hospital kitchen. Specimens collected from surveys of the kitchen environment were examined and also rectal swabs from cattle, pigs, and chickens immediately after slaughter. The sources of these strains have been fully described elsewhere (Cooke, Ewins and Shooter, 1969; Cooke *et al.*, 1970; Shooter *et al.*, 1970).

The frequency of isolation of different colicine types of *E. coli* from the patients is shown in table VI. Types 2 and 4 accounted for 40 per cent. of the strains that could be colicine typed. Hospital patients had in their faeces more strains of type 2 and 4 than did people outside hospital ($P = < 0.01$).

It was also observed that the longer the patients stayed in hospital, the more

prominent became colicine types 2 and 4 in their faeces. These two types accounted for one-third of all typable strains isolated from patients who had

TABLE VII
Prevalence of colicine types of E. coli in patients related to length of stay in hospital

Colicine types	Number of strains of the stated colicine types (and percentage of all typable strains) isolated from patients who stayed in hospital for	
	less than 20 days	more than 20 days
2 and 4	22 (30·6)	42 (47·7)
Others	50 (69·4)	46 (52·3)
All typable strains	72 (100)	88 (100)

an in-patient stay of less than 20 days, whilst they comprised nearly half of all typable strains isolated from patients in hospital for more than 20 days (table VII).

TABLE VIII
Frequency of colicine types 2 and 4 in E. coli from various sources

Source	Number (and percentage) of			
	specimens examined	strains examined	colicinogenic strains	strains types 2 and 4
Rectal swabs of:				
cattle	37	51	16 (31)	14 (27)
pigs	11	63	25 (40)	13 (21)
chickens	18	69	49 (71)	23 (33)
Meat on arrival at hospital:				
beef	28	20	4 (20)	2 (10)
pork	17	10	2 (20)	0
chicken	30	61	49 (80)	16 (26)
other	47	45	8 (18)	2 (4)
Food and utensils	236	106	42 (40)	6 (6)
Kitchen environment	251	130	17 (13)	0
Food in ward	731	65	9 (14)	0
Patients*	1401	429	136 (32)	54 (13)
Patients†	125	142	51 (36)	20 (14)
Normal persons	93	144	61 (42)	8 (6)

* Strains of defined serotype isolated during 12 mth.

† All strains isolated during 3 mth.

Many *E. coli* of colicine types 2 and 4 were found in the animals examined and in the chickens delivered to the hospital. We also found these strains in a survey of food and utensils in the kitchen, but not in the kitchen environment or the food as it was being served (table VIII).

DISCUSSION

The criteria for a useful method of typing in epidemiological work are that the results should be reproducible, that a high proportion of strains should be typable, and that it should provide an adequate subdivision of types. The results obtained from colicine typing showed a good degree of reproducibility. In only five of 42 instances in which the same serotype was isolated in replicate isolates was a variation in colicine type observed. In three instances this variation may have been due to the acquisition of a *col* factor by a strain whilst resident in the bowel, because the new colicine type observed in these instances differed from the original by a gain in inhibition of more than one indicator. Replicate isolates of the same serotype but different colicine type do not necessarily indicate instability of colicine type. It may be that such isolates represent serologically distinct strains sharing a common antigen, a difference that might have been detected if complete serological identification had been made. In this study serological characterisation of strains was by determination of the O antigen only.

Of 20 strains tested after 6 months' storage, two showed a variation in type. This lack of reproducibility could not be due to an instability of the indicator strains, because serial isolates from patients typed simultaneously showed a remarkable constancy of type. It might be due to a variation in potency; McGeachie and McCormick (1967) observed such a variation when strains were re-tested by the same methods of preparation of medium and colicine extraction.

The differences between the results from different methods of detecting colicine production are not surprising. A similar lack of complete correlation between *in-vitro* colicine assay methods has been reported by other workers (Hentges and Freter, 1962; Branche *et al.*, 1963). The discrepancy in the results from the two typing methods is also to be expected because certain colicines—such as colicine I—diffuse slowly (Ishikura *et al.*, 1969) and are best detected after 48–72 hours' incubation whilst others are labile (Mennigmann, 1965; Kubota, *et al.*, 1969) or are inactivated by concomitantly produced inactivators (Foulds and Shemin, 1969), and are therefore best detected after 24 hours' incubation.

The work reported here, although it confirmed the stability of colicine type *in vitro* and *in vivo* and its reliability as an epidemiological marker, showed that it was of limited value mainly because only a third of the strains were colicine producers and because many of the strains fell into two groups. The low incidence of colicinogenicity observed in this study was not due to the choice of shigellae as indicators. Other workers using *E. coli* as indicators reported similar figures (Fredericq, 1946; Linton, 1960).

Although the findings of some previous workers suggest a relationship between colicine type and serotype, we found no correlation between colicine production and serotype—a finding similar to that of Vosti (1968).

Although colicine typing had several deficiencies as an epidemiological marker it was occasionally of value in assessing the significance of repeated

isolations of the same serotype from individual patients and of the simultaneous occurrence of a common serotype in more than one patient in the ward. It was also of value in indicating possible sources of *E. coli* in the bowel of hospital patients. The finding in the epidemiological investigations of the excess of colicine types 2 and 4 compared with normal persons was unexpected. As we had previously obtained evidence that strains of *E. coli* of animal origin were being fed to our patients in food, we colicine typed strains that we had obtained from animals. In these also we found many types 2 and 4. *E. coli* of animal origin will reach the human population only when cross-contamination occurs in the kitchen and the food is kept under conditions in which organisms can multiply. Although these investigations do not precisely define the route by which *E. coli* reaches the patient's bowel, they do indicate that animals may be a source. Colicine typing may be useful in other investigations of this type.

SUMMARY

Colicine production and sensitivity were determined for strains of *E. coli* of defined serotype isolated from patients in a medical ward. The strains could be assigned to 19 colicine types as determined by production patterns. No correlation was observed between colicine production or sensitivity and serotype. Colicine typing proved to be stable and hence reliable as an epidemiological marker. However, only one-third of the strains of *E. coli* were typable. We used this method in an epidemiological investigation and found some evidence that *E. coli* of animal origin were reaching hospital patients via food.

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