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Escherichia coli in gastroenteritis of children in Auckland, New Zealand

BY P. N. GOLDWATER,* K. A. BETTELHEIM†
AND R. B. ELLIS-PEGLER*

* *Infectious Disease Unit, Auckland Hospital, Auckland, New Zealand*
† *Serology Department, National Health Institute, Wellington, New Zealand*

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SUMMARY

A study of stool *Escherichia coli* in 60 children with gastroenteritis and 18 control children was carried out in Auckland, New Zealand in 1977. Toxigenic strains, heat labile and heat stable, predominated in the stools of only three children, all of whom had concomitant rotavirus. Classical enteropathogenic *E. coli* (EPEC) were found in patients and controls. Only one patient had many EPEC in the stool (086.H2), they were variably toxigenic and rotavirus was also present. No toxigenic serotype was isolated. Two potential pathogens were sometimes found. Overall there was no evidence for a substantial causative role for disease producing *E. coli* in these children.

INTRODUCTION

Many organisms are responsible for gastroenteritis in childhood. Rotavirus, salmonellae, shigellae, campylobacter and giardia are widely accepted pathogens and are those most commonly incriminated.

Classical enteropathogenic *E. coli* serotypes (EPEC) have been causally linked with this syndrome since the 1940s but the pathogenesis of such infections still remains uncertain, and their relevance much debated. With the realization that some strains of *E. coli* can produce one or more of a number of toxigenic factors (Sack, 1975; Ørskov *et al.* 1976; Evans, Evans & Du Pont, 1977; Klipstein *et al.* 1978) serotyping has seemed less important. However a statistical association between diarrhoeal disease in children in the community and EPEC isolation has recently been confirmed in sporadic cases of gastroenteritis (Gurwith *et al.* 1978), and a survey of serotypes of *E. coli* from a variety of sources (Bettelheim, 1978) demonstrated that certain 'OH' serotypes are much more commonly associated with infantile gastroenteritis than others.

There appear to be genuine geographical variations in the relative contributions of the various heat stable, heat labile and dual toxin producing enterotoxigenic *E. coli* (ETEC) strains, (Merson *et al.* 1979; Back *et al.* 1980). Differences in laboratory assessment and intrinsic variation in toxigenicity (Echeverria *et al.* 1977) might also affect their relative contributions on occasion. It is still not possible to determine the human pathogenicity of EPEC with routine *in vitro* techniques so that their pathogenic role when isolated from the stools of individual

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patients necessarily remains uncertain. All these factors conspire to make the assessment of the role of *E. coli* in gastroenteritis difficult, and comparisons uncertain.

This study forms part of a larger aetiological assessment of gastroenteritis in children carried out during two winter months in Auckland, New Zealand, in 1977 and reported elsewhere. (Goldwater, 1979). Here the potential contributions of *E. coli* are explored in greater detail.

MATERIALS AND METHODS

These are described in detail elsewhere (Goldwater, 1979) and summarized here. Sixty children with gastroenteritis (< 6 years old) admitted to the Infectious Disease Unit, Auckland Hospital during June and July 1977 were studied. Eighteen children (< 6 years old) admitted to the same unit for other than gastroenteritis served as hospital controls. A stool passed within 24 h of admission was examined by immuno-electronmicroscopy for rotavirus, by conventional techniques for isolation of *Salmonella* and *Shigella*, and stained for *Giardia lamblia* (1 % methylene blue). No attempt was made to isolate campylobacter. Specimens were streaked on to MacConkey agar and after overnight incubation, 10 lactose fermenting colonies, presumptive *E. coli*, were selectively picked and stored on nutrient (Columbia) agar slopes for immediate enterotoxin testing in pools and later biochemical identification and serotyping. Assay for heat stable toxin (ST) was carried out by the suckling mouse assay (Dean *et al.* 1972). Mean gut:body weight ratios > 0·0700 were classified as positive. Assay for heat labile toxin (LT) was carried out by the Y-1 adrenal cell assay (Sack & Sack, 1975). 'O' and 'H' serotyping was performed by methods previously described (Chandler & Bettelheim, 1974; Meekin, Bettelheim & Bacon, 1979) and carried out at the National Health Institute, Wellington, New Zealand. When confirmed biochemically as *E. coli*, two colonies were later arbitrarily selected for serotyping. Where toxin positive and negative isolates were present, toxin positive isolates were usually discriminatively selected for serotyping. Strains were stored after the initial phase of the study: when an attempt 2 years later was made to serotype all the colonies picked from stools which contained any toxigenic strains, many had died. This explains our failure to serotype more than the original two strains from many of these particular specimens.

RESULTS

The patients and controls are divided into two groups (Table 1); with (Group 1) and without (Group 2) toxin positive isolates, the former comprising only eight children and the latter, 70. Within these groups they are subdivided into smaller groups: those with diarrhoea and stool rotavirus (1 A and 2 A, three and 38 children respectively), those with diarrhoea without stool virus (1 B and 2 B, two and 17 children respectively) and the controls (1 C and 2 C, three and 15 children respectively). The results are tabulated (Tables 2 and 3) and discussed in relation to these groups.

No patient had *G. lamblia* in the stool. Two patients had *Shigella* species and one *S. enteritidis* ser. typhimurium in the stool. Three control patients had rotavirus in their stool.

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Table 1. *Patient and control groups*

		Number of patients Toxin	
		+ve (Group 1)	−ve (Group 2)
Gastroenteritis			
Rotavirus	(A)	3 (1 A)	38 (2 A)
No rotavirus	(B)	2 (1 B)	17 (2 B)
Controls	(C)	3 (1 C)	15 (2 C)
Total		8	70

Figures and letters in brackets refer to groupings defined and used in the Results section.

(1) *Patients with toxin positive isolates (Group 1)*

(*a*) *Toxin testing*

All strains classified as ST+ produced mean gut:body weight ratios between 0·070 and 0·080. They thus fall below levels accepts by most as definitely positive (< 0·080). At best they can thus only be classified as having some lesser degree of toxigenicity (Klipstein, Engert & Short, 1977; Goldwater, 1979).

Toxin positive strains were found in a small number of patients from each sub-group (Table 2). In all three patients from Group 1 A, (26, 51, 86) the toxigenic isolates, either LT or ST, predominated among the faecal *E. coli*. In two patients some strains were LT+ST+ (26I and 86 E, F, H, J), while in the five other children in Group 1 (28, 51, 65, 78, 88) only strains either LT+ or ST+ were found, i.e. none had LT+/ST+ strains and these toxin positive strains in any individual specimen were never the majority. In particular this was true of the two patients in Group 1 B (diarrhoea without stool virus), i.e. only 1/9 ST+ strains in patient 28 and 2/10 ST+ strains in patient 65. One patient (91) had klebsiella isolated, three of the four tested isolates being ST+.

(*b*) *Serotyping*

Although unserotyped strains from this group of patients clearly diminishes the information base (only 21/68 picked colonies were serotyped) no patient was found with a toxigenic serotype (Report, 1980). With the exception of patient 51, all had differing stool serotypes with no particular relationship to toxigenicity. LT+ serotypes isolated were 0145 . H 11, 015 . H 12 and 089 . H 21, ST+ serotypes Ont. H 9 and 08 . Hnt, while a single Ont . H 11 isolate was LT+/ST+. Patient 51 differs: only 086 . H 2 was isolated, this is a classical EPEC, and the individual 086 . H 2 strains were either LT+, ST+ or LT−/ST−.

(2) *Patients with toxin negative isolates (Group 2)*

Serotyping

A wide range of serotypes was found in all groups (Table 2). Several EPEC strains were found within Group 2 A. There were patients with single EPEC strains 055 . H 34, 018ac . H 7, 086 . H 2, 0127 . H 6 and 0127 . H 1, each accompanied by a different non EPEC strain, and two patients with 0127 . H 6 as their two selected

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Table 2. *Group 1 with toxin positive pools: serotypes isolated and their relationship to toxigenicity*

Patient no.	Picked colonies	Toxin ST	Toxin LT	Serotype
Group 1A – patients with stool rotavirus				
26	A, B, H, J	–	+	nd
	C	–	nd	nd
	D, F	+	–	nd
	E	nd	nd	nd
	G	–	–	nd
	I	+	+	nd
51	A, B	+	–	086.H2
	D, E, G	–	–	086.H2
	C, F	+	–	nd
	I, J	–	+	086.H2
	H	nd	+	086.H2
86	A	–	+	0145.H11
	B, C, D	–	+	nd
	E	+	+	Ont.H11
	F, H, J	+	+	nd
	G, I	–	–	nd
Group 1B – patients without stool rotavirus				
28	C	+	–	nd
	F	–	–	Ont.H2
	H	–	–	Ont.H11
	A, D, E, G, I, J	–	–	nd
65	E, F	+	–	Ont.H9
	A, B	–	–	R.H–
	G, I	–	–	068.H–
	C, D, H, J	–	–	nd
Group 1C – controls				
78	I	–	+	015.H12
	J	–	+	089.H21
	A, C, G	–	+	nd
	H	–	–	nd
	B, D, E, F	nd	nd	nd
88	H	+	–	08.Hnt
	C, D, E, F, I, J	–	–	nd
	A, B, G	nd	nd	nd
91	All strains *Klebsiella*			
	C, D, J	+	nd	nr
	G	–	nd	nr
	A, B, E, F, H, I	nd	nd	nr

nt denotes non typable; – indicates absent; nd denotes test not done; R denotes a rough strain; nr denotes not relevant.

isolates. Within Group 2B there was one patient with a single EPEC strain 0128.H–, while EPEC 018ab.H– and 018ac.H– were the two typed isolates of one patient in the control group (Group 2C). Strains known to be common commensals were found in stools from all three groups of patients but were a minority.

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Table 3. *Group 2 with toxin negative pools: serotypes isolated*

Group 2A – patients with stool rotavirus
Ont Hnt. (2); Ont H–. (10); Ont H4. (1); ONT H6. (1); Ont H10. (1); Ont H14. (1); Ont H16. (1); Ont H18. (2); Ont H38. (2); ONT H41. (1).

03 H2. (1); 08 H–. (1); 0 rel 8 H–. (1); 011 H8. (1); 015 H18. (1); 018 ac H7. (1); 021 H–. (1); 055 H34. (1); 063 H7. (1); 068 H9. (1); 068 H–. (1); 075 H5. (2); 083 H4. (1); 086 H2. (1); 0H3 H21. (1); 0127 H6. (5); 0127 H21. (1).

Group 2B – patients without stool rotavirus
Ont Hnt. (5); Ont H–. (6); Ont H31. (2); RH–. (2).

02 H6. (2); 05 H11. (1); 07 H–. (1); 08 Hnt. (2); 015 H18. (1); 073 H–. (1); 0128 H–. (1).

Group 2C – controls
Ont Hnt. (1); Ont H–. (2).

02 H1. (1); 06 H1. (1); 08 H19. (1); 016 H6. (3); 018 ab H–. (1); 018 ac H–. (1); 070/084 H21. (1); 070/084 R. (1); 092 H33. (1); 0151 H11. (2).

Abbreviations are as in Table 2 and rel. denotes related to.

There were many untypable O and H strains in all six groups. No single serogroup dominated the faecal flora of any particular diarrhoeal group, nor all of the groups, during the course of this investigation.

DISCUSSION

The large number of O nt and H nt *E. coli* strains reflects the largely unserotyped state of New Zealand *E. coli*. It is likely that the local flora is antigenically distinct from that of other parts of the world: 092 . H33 for example, found in one control patient is rare other than in New Zealand.

The three patients with predominantly toxigenic strains in their stools in our study all had rotavirus as well; they also had mixtures of toxin types. In the group with diarrhoea who did not have stool rotavirus, in which one might have predicted a possible contribution from ETEC, only two patients had toxigenic strains. They were only one and two strains respectively of the nine and ten tested from those two patients and both were only ST producers. Classical studies have demonstrated that causative bacterial pathogens in the faeces of patients with diarrhoea, e.g. EPEC (Taylor, 1966), *V. cholerae* (Gorbach *et al.* 1970) and ETEC (Gorbach *et al.* 1971) usually predominate in the stool and are present in high concentration.

As the assessment of heat stable toxin only produced *in vivo* mouse abnormalities which we classified as 'mildly toxigenic' and would probably be classified as negative by many workers (e.g. Whipp, Moon & Lyon, 1975), the potential relevance of toxin positive isolates in this environment is reduced even further. Finally we failed to find any of those serotypes which others commonly find to be ETEC. (Report, 1980).

E. coli isolates from sporadic cases of gastroenteritis throughout New Zealand submitted to the National Health Institute, Wellington and tested for toxin production by the techniques we used and the Vero cell test for cyto-toxin (Konowalchuk *et al.* 1978) also reveal only 5–7 % to be toxigenic (Bettelheim, to

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be published). This is broadly similar to our findings and also argues for a minimal role for these organisms in New Zealand.

Predominantly negative results were obtained from the serotyping in terms of potential causative contributors to gastroenteritis. A few EPEC isolates were identified in all the diarrhoea groups and the controls. Only one patient (51) had many EPEC in the stool, they were mostly toxin producers as well, and rotavirus was also present. In the toxin negative group with diarrhoea and without stool rotavirus, again a group to which EPEC might have contributed, only one EPEC strain (0128 . H–) was isolated. On even the most liberal interpretation of this data, classical EPEC strains, causing disease by uncertain mechanisms, were rarely involved.

Like others, (Echeverria *et al.* 1977; Bishop *et al.* 1979) we quite often found two potential pathogens in stools. Given the frequency of this finding, interaction between gut pathogens may still turn out to be important. We accept that viral infection may be complicated by bacterial infection in the respiratory tract (Louria *et al.* 1959; Schwarzmann *et al.* 1971). There seems no special reason to deny the analogous possibility in the gut.

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