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Escherichia coli in gastroenteritis of children in London and Jamaica

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SUMMARY

The jejunal and stool flora of children with gastroenteritis in London and in Jamaica was examined. Although bacterial colonization of the small bowel was commonly detected, it was unusual to find the same serotype of *E. coli* in both jejunum and stool, and none of the jejunal strains of *E. coli* produced either heat-labile or heat-stable enterotoxin. Some strains of *E. coli* causing infant gastroenteritis are neither toxigenic nor invasive, and other mechanisms must be sought to account for their pathogenicity.

INTRODUCTION

Until recently the aetiology of most of the common illnesses categorized as acute gastroenteritis remained unknown. Two main lines of advance have now become apparent, one concerned with the role of recently recognized viral agents, increasingly implicated in the aetiology of infant diarrhoea, and the other with the pathogenesis of bacterial diarrhoea, especially that associated with strains of *E. coli*. In the past evidence of pathogenicity of *E. coli* strains has rested mainly on epidemiological data relating particular serotypes to outbreaks of infant gastroenterities (Gangarosa & Merson, 1977). More recently, much work has been published to show that strains of *E. coli* may produce enterotoxins and this property has been correlated with pathogenicity in travellers' diarrhoea in adults (Gorbach *et al.* 1975; Merson *et al.* 1976) and in some (Guerrant *et al.* 1975; Sack *et al.* 1975) but not other (Echeverria, Blacklow & Smith, 1975; Gross, Scotland & Rowe, 1976) studies of childhood and infant gastroenteritis. Doubt has been cast on the value of serotyping in sporadic cases of infant diarrhoea. On the other hand enterotoxin production in laboratory conditions may not be as closely related to pathogenicity in man as is the case in cholera, and the importance of other pathogenic factors needs to be delineated; for example, the ability of potential pathogens to adhere to bowel mucosa, and to establish themselves in the bowel.

Colonization of the small bowel is a notable feature in cholera, but little is known of its frequency, extent and significance in infant gastroenteritis. This study gives an account of the Enterobacteriaceae isolated from the faeces and jejunum

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in two groups of children from Kingston, Jamaica, and London, England. A detailed account of the clinical and biochemical features of their illnesses and of their stool and jejunal flora is given elsewhere (Ellis-Pegler, Higgs & Lambert, 1978). Here the relation between small bowel colonization, enterotoxin production and disease is explored.

METHODS

Faeces or upper small bowel luminal fluid was diluted and cultured as previously reported (Ellis-Peger, Crabtree & Lambert, 1975). From those McConkey plates at the higher dilutions, where discrete colony forms could be recognized, ten lactose fermenting colonies, including at least one of each colonial type present, were picked and subcultured. These colonies were identified by routine biochemical methods. From each specimen five of the cultures identified as *E. coli* biochemically, including at least one of each of the different colonial types initially recognized, were subcultured on nutrient agar in bijoux bottles. These were kept at room temperature before sending to the Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale, London.

Swabs from undiluted (or from 1/10 dilutions of the frozen) specimens were cultured directly onto deoxycholate-citrate agar (D.C.A.) and potential *Salmonella* and *Shigella* organisms similarly referred to the Central Public Health Laboratory.

These *E. coli* cultures were serotyped at Colindale according to the accepted international scheme using antisera for O groups O1 to O164 and for flagella antigens H1 to H56 (Ørskov & Ørskov, 1975).

Total concentrations of Enterobacteriaceae represent the total counts of lactose fermenting (and where present, non-lactose fermenting) colonies. These were counted from a dilution on McConkey medium on which discrete colonies could be readily discerned.

Enterotoxin testing

One representative culture of each *E. coli* serotype found in each small bowel specimen was tested for the production of heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST). The infant mouse test was used for the detection of ST (Dean *et al.* 1972) and the CHO cell (Guerrant *et al.* 1974) and Y1 cell (Donta, Moon & Whipps, 1974) tests were used to detect LT.

RESULTS

Four out of 11 and five out of nine of the children fully investigated in Jamaica and England respectively had at least 10^2 Enterobacteriaceae per ml of small bowel lumen fluid during the acute phase of diarrhoea (Table 1). Jejunal specimens were taken from another six children (three from Jamaica and three from England), and were tested only by routine laboratory screening procedures for enteropathogenic *E. coli* but none were identified. One of these patients had a low count of *Alkaligenes faecalis* in the jejunal fluid.

E. coli was found in the small bowel lumen of seven children (J11, J19, 260, 273,

Table 1. E. coli *and other Enterobacteriaceae in small bowel and/or faeces of infants with gastroenteritis in London and Jamaica*

Code	Small bowel strains	Concentration of bacteria/ml (log_{10})	Faecal strains	Concentration of bacteria/ml (log_{10})
		Jamaica		
J11	O111.K58.H2 O15.K?H1 *Enterobacter* sp.	4·1	O111.K58.H2	5·5
J19	O21.K?H?	2·7	O18ab.K?H14 O153.K?H7 *Klebsiella* sp.	6·0
J14	*Enterobacter* sp.	2·5	O unident.H- O21.K?H? *Klebsiella* sp.	3·2
J55	(*a*) *Klebsiella* sp.	3·0	O12.K5.H12 O unident.H12 O unident.H33 O3.K?H4 O15.K?H1 *Klebsiella* sp.	8·2
	(*b*) No Enterobacteriaceae			
J5	No Enterobacteriaceae		O111.K58.H2	9·2
J32	(*a*) No Enterobacteriaceae		O114.K90.H21 O127.K63.H40 *Proteus* sp.	8·3
	(*b*) No Enterobacteriaceae			
J39	(*a*) No Enterobacteriaceae		O unident.H10 O86.K?H18 *Klebsiella* sp.	9·1
	(*b*) O unident.H10	2·7		
J27	No Enterobacteriaceae			
J9	No Enterobacteriaceae		O unident.H12 O unident.H2 O83.K?H28 *Klebsiella* sp.	7·9
J25	No Enterobacteriaceae		O80.K?H27 *Klebsiella* sp.	8·2
J36	No Enterobacteriaceae		O39.K?H-	8·6
		England		
260	O111.K58.H2	5·7	O111.K58.H2	8·9
273	O3.K?H2	3·5	O3.K?H2	4·8
221	O22.K13.H1 *Citrobacter* sp.	4·3	O111.K58.H2 O unident.H33	9·2
267	O4.K?H5	3·6	O unident.H7 O2.K?H2 02.K?H5 *Salmonella agona*	4·7
280	O75.K?H-	5·0	O90.K?H25 O90.K?H-	8·8
227	No Enterobacteriaceae		O125.K70.H11	8·1
237	No Enterobacteriaceae		O unident.H9 O125.K70.H6 *Salmonella typhimurium*	8·0
259	No Enterobacteriaceae		O75.K?H5	2·3
238	No Enterobacteriaceae		O75.K?H5	8·1

Three patients were intubated in the acute stage (*a*) and in convalescence (*b*).

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Table 2. *Jejunal and stool enterobacteria in children with acute gastroenteritis*

(Total with Enterobacteria in jejunum, 9; total with enteropathogenic *E. coli* (E.P.E.C.) in stool and/or jejunum, 8).

Jejunum		Stool	No.
E.P.E.C.	Same	E.P.E.C.	2
Non-E.P.E.C.	Same	Non-E.P.E.C.	1
Non-E.P.E.C.		E.P.E.C.	1
Non-E.P.E.C.		Salmonella	1
Non-E.P.E.C.	Different	Non-E.P.E.C.	1
No *E. coli*		E.P.E.C.	5
Other Entero-bacteria		Non-E.P.E.C.	2

Table 3. *Jejunal flora in acute gastroenteritis*

	Jamaica	U.K.	Total
No.	13	12	25
$> 10^3$ Aerobes	10	10	20
$> 10^4$ Aerobes	8	7	15
$> 10^3$ Anaerobes	5	5	10
$> 10^4$ Anaerobes	3	4	7

221, 267, 280). Of these, two (J11 and 260) had a recognized enteropathogenic serotype in both small bowel and in faeces. In both patients the concentrations of this organism (O111.K58.H2 in both) were the highest small bowel concentration of Enterobacteriaceae recorded for each geographical group. A third child (273) had the same non-enteropathogenic serotype (O3.K?H2) in both sites. Two more children (221 and 267) had recognized pathogenic organisms (*E. coli* O111.K58.H2 and *Salmonella agona*) in their stools (along with other non-enteropathogenic *E. coli* serotypes) while their small bowels contained different serotypes. The Salmonella species was not isolated from the small bowel (267). The remaining two patients (J19 and 280) with *E. coli* in the small bowel lumen also had differing stool and small bowel serotypes.

Two more patients both from Jamaica (J14 and J55) had other Enterobacteriaceae species in the small bowel lumen, with no faecal *E. coli* belonging to recognized enteropathogenic serotypes.

None of the remaining patients had small bowel Enterobacteriaceae though five of them had enteropathogenic *E. coli* serotypes in their stools (J5, J32, J39, 227, 237). Patient J39, re-intubated in convalescence when free of diarrhoea, had a small bowel serotype which had been present only in the faeces during the acute illness. Two other patients (J55, J39), re-intubated in convalescence, showed no Enterobacteriaceae in the jejunal fluid. These relationships are summarized in Table 2 and a summary of total aerobic and anaerobic jejunal flora in the whole group of 25 children is given in Table 3.

None of the *E. coli* from small bowel specimens produced enterotoxin (ST or LT) detectable by any of the three methods used.

DISCUSSION

Production of heat-labile and heat-stable toxin by *E. coli* and other Enterobacteriaceae has now been studied by a wide variety of methods and the incidence of toxigenic strains has varied greatly in different studies. Workers impressed with the relationship between enterotoxigenicity and illness have doubted whether serotyping is now a valid method of identifying pathogenic strains since enterotoxin production, which is plasmid-mediated (Smith & Lingood, 1971) may not be a strain-specific property. On the other hand, good epidemiological evidence has been adduced to link the 'classical' infantile enteropathogenic serotypes with pathogenicity and negative toxin tests have been obtained in several studies of diarrhoeal disease in childhood and in a survey of strains firmly associated with well-defined epidemics in Britain (Echeverria *et al.* 1975; Gross *et al.* 1976).

If heat-labile enterotoxin, acting in a manner similar to that of cholera, is an important factor in the pathogenesis of infant gastroenteritis, it should be possible to demonstrate its presence in the small bowel during the acute illness, but this aspect of pathogenesis has seldom been studied. Thomson (1955) showed that in babies with gastroenteritis associated with enteropathogenic serotypes of *E. coli*, the small bowel lumen was heavily colonized with these organisms. In control subjects, convalescents and infants with gastroenteritis of unknown cause, *E. coli* were found rarely and then in small number, in the small bowel. Sack *et al.* (1975) intubated 19 Apache children on 21 occasions. Eleven of the children showed jejunal counts of more than 10^3/ml, but only one enteropathogenic serotype and one enterotoxigenic strain were isolated from the jejunal contents. Our findings were similar to theirs and showed that colonization of the small bowel was fairly common in both temperate and tropical climates but uniform colonization of stool and small bowel by a single serotype was rare. Moreover, none of the jejunal strains of *E. coli*, whether of recognized enteropathogenic serotype or not, produced enterotoxin detectable by any of the three methods by which they were tested. Three of the jejunal strains were of the same serotype as the dominant stool strain, and two of these belonged to O group O111, a 'classical' enteropathogenic serogroup frequently identified in outbreaks of infant gastroenteritis.

Studies of small bowel flora are difficult to interpret since transient and partial colonization may easily be missed by one examination at a single site. None the less, the negative toxin tests on jejunal isolations of *E. coli* must throw further doubt on the idea that enterotoxin production is the only pathogenic factor in infant gastroenteritis. Strains which produce enterotoxin may also require other properties such as mucosal adherence (McNeish *et al.* 1975) and colonizing capacity (Evans *et al.* 1975) before they are able to cause diarrhoea. Furthermore, some *E. coli* cause diarrhoea by mechanisms which do not involve enterotoxin. One such mechanism is already known and depends on the ability of some strains to invade the intestine epithelium in a Shigella-like manner (Dupont *et al.* 1971).

It has also been suggested that the classical infantile enteropathogenic serotypes may cause diarrhoea by mechanisms as yet undescribed but not involving enterotoxin production or invasiveness (Rowe, Scotland & Gross, 1977). The isolation of such serotypes from the jejunal fluid, sometimes in pure culture, provides supporting evidence for this concept.

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