JEJUNAL MICROBIAL FLORA OF SOUTHERN INDIAN INFANTS IN HEALTH AND WITH ACUTE GASTROENTERITIS

M. J. ALBERT, P. BHAT, D. RAJAN, P. P. MAIYA, S. M. PEREIRA,
M. MATHAN AND S. J. BAKER*

*Wellcome Research Unit, Departments of Child Health and Pathology,
Christian Medical College and Hospital, Vellore 632 004, Tamil Nadu, India*

ACUTE gastroenteritis in infancy is an important cause of morbidity and mortality in many parts of the world (Gordon, 1971). Recent studies from this hospital (Maiya *et al.*, 1977) have shown that in southern India, it is most frequently due to infection with a variety of bacterial pathogens, or to infection with rotavirus, but in some cases the aetiology remains obscure. In order to obtain more information on aetiology and pathogenesis, the bacterial flora in the lumen of the jejunum of infants with acute gastroenteritis was studied and compared with that of control subjects.

MATERIALS AND METHODS

Subjects. Twenty-eight infants, aged 2–12 months, who were admitted to hospital with acute diarrhoea of 1–3 days' duration were studied. The infants belonged to parents of low socio-economic status. Ten infants of similar age and socio-economic status without any evidence of gastrointestinal disease were studied as controls. Consent of the parents was obtained for the participation of their children in the study. All the children were receiving breast-milk feedings supplemented with cows' milk. None of the patients or the controls had received any antimicrobial therapy before the investigation.

Specimens. Specimens of jejunal juice were collected by introducing a sterile radio-opaque polyvinyl tube, with the aid of a fluoroscope, an image intensifier and a television chain. When the tube reached the upper jejunum just below the duodenojejunal flexure, intestinal juice was aspirated. The first few millilitres of the aspirate were discarded. The next 2–5 ml of juice were placed in a sterile container and processed immediately.

Specimens were obtained as soon as possible after the patients were admitted to hospital. In severely dehydrated patients, the specimens of jejunal juice were obtained after rehydration, always within 24–48 h of admission. The specimens from patients were obtained when either nothing or merely sterile electrolyte solution had been given by mouth. Specimens from the controls were obtained at least 6 h after the last feed.

A fresh stool specimen was also collected in a sterile container as nearly as possible to the time of intubation. This was also processed immediately.

Microbiological methods. The jejunal juice was diluted with sterile saline to produce dilutions ranging from 10^1 to 10^8 . The faecal sample was suspended in sterile saline to give a concentration of 0.1 g per ml and this material was similarly diluted over the range 10^1 to 10^8 ; 0.1-ml portions of the dilutions were cultured on various media. The details of the media, the methods of incubation and the various groups of organisms identified are

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* Present address: Department of Medicine, St Boniface Hospital, Winnipeg, Manitoba, Canada R2H 2A6.

reported elsewhere (Bhat *et al.*, 1972). Bacterial counts are expressed as the logarithm to the base of 10. The sera used for detection of enteropathogenic *Escherichia coli* (EEC) covered the following serotypes—0111:B4, 055:B5, 026:B6, 086:B7, 0127:B8, 0124:B17, 0125:B15, 0126:B16, 0128:B12 and 0119:B14. Stools were examined for rotavirus particles by the method of Flewett, Bryden and Davies (1974).

RESULTS

The bacteriological findings in the jejunal samples are shown in table I. Samples from the control infants had total viable bacterial counts that ranged from 1·8 to 5·7 (expressed as the $\log_{10}$ of the count per ml of juice). In the samples from infants with diarrhoea, the total counts ranged from 2 to 8·6 log (expressed as the $\log_{10}$ of the count per ml of original material). Enterobacteria and lactobacilli were present in these samples in significantly greater numbers than in samples from the controls ($P < 0.05$). Enterococci and bacteroides organisms were present respectively in five and six of the specimens from patients, but were not found in specimens from the controls. The total Gram-negative aerobic rods in the jejunal juice of controls and patients is shown diagrammatically in the figure. A detailed analysis of the Gram-negative aerobic rods found in the jejunal samples, and the presumed pathogens grown

TABLE I
Aerobic and anaerobic microflora cultured from jejunal aspirates of 10 control subjects and 28 patients with diarrhoea

Organisms	Numbers of control subjects positive	Mean counts* in control subjects	Ranges of counts* in control subjects	Numbers of patients positive	Mean counts* in patients	Ranges of counts* in patients
Enterobacteria†	4	2·7	2·0–3·7	16	5·3	3·0–8·4
Pseudomonads and other non-fermenting Gram-negative rods	4	3·0	2·0–4·3	9	4·3	2·3–7·3
Neisseriae	3	3·3	2·6–4·5	4	4·0	2·1–5·2
Staphylococci including micrococci	2	4·1	3·3–4·8	3	3·5	2·6–4·6
Enterococci	0	...	...	5	4·4	2·8–6·9
Streptococci	9	4·5	2·3–5·7	22	4·9	2·7–7·6
Lactobacilli†	5	2·6	1·5–4·2	12	4·3	1·7–6·4
Total aerobes	10	4·6	1·9–5·7	27	5·3	2·0–8·6
Yeasts and yeast-like organisms	4	2·1	1·0–3·4	16	3·4	2·0–5·9
Bifidobacteria	5	2·8	1·0–4·4	6	4·2	1·8–6·5
Bacteroides	0	...	...	6	4·1	2·3–7·6
Fusobacteria	4	2·0	1·0–3·3	2	1·2	1·0–1·3
Veillonellae	5	3·7	2·7–4·6	12	3·5	1·0–5·0
Anaerobic streptococci	4	4·6	3·8–5·4	17	5·1	2·7–7·2
Total anaerobes	8	3·9	2·0–5·5	23	5·0	1·8–7·6
Total bacteria	10	4·7	1·9–5·7	27	5·6	2·0–8·6

* Values are expressed as $\log_{10}$ bacterial count per ml of fluid.

† The differences between controls and patients are significant at the 5% level.

FIGURE.—The total number of Gram-negative aerobic rods in the jejunal juice of infants with gastroenteritis and control infants.

from the stools, are shown in table II. In eight patients (nos. 1–8) the same presumed bacterial pathogen was recovered from both the jejunal juice and the stool. Three patients (nos. 9–11) had presumed pathogens in the stool, but these organisms were not found in the jejunal juice, even though it contained other Gram-negative aerobic rods. In seven patients (nos. 12–18) Gram-negative aerobic rods were present in the jejunum, but no presumed pathogens were isolated from the jejunum or stools. In four patients (nos. 19–22) no Gram-negative aerobic rods were isolated from the jejunum, but in three a pathogenic *E. coli* was isolated from the stool. In none of these 22 infants was rotavirus detected.

In five of six patients (nos. 23–28) in whom rotavirus was present in the stools, no Gram-negative aerobic rods were found in the jejunal juice, but in one (no. 28) pseudomonads were present with a low viable count of 2.7 ($\log_{10}$ per ml). In one of these patients (no. 27) EEC 086:B7 was present in the faeces with a count of 7.4 ($\log_{10}$ per ml).

DISCUSSION

The bacteria found in the jejunal juice of the control subjects in this study are comparable with those found by Challacombe, Richardson and Anderson (1974a) in normal English children who had an "oral" type of flora, and in the Australian children studied by Bishop, Barnes and Townley (1974a), who also had a mainly "oral" type of flora although a few children harboured coliforms. However, in these earlier studies, bacteria were present in only half of the subjects, half the samples being sterile, whereas in the present study all the control subjects had bacteria in their jejunal juice. The greater colonisation of the jejunum in south Indian infants is in conformity with studies in adults published previously (Bhat *et al.*, 1972).

TABLE II

Gram-negative aerobic bacilli isolated from the jejunum or faeces of 22 patients with gastro-enteritis in whom rotavirus was not found

Patient no.	Organisms in jejunum	Count (log ₁₀ per ml) in jejunum	Presumed pathogens in faeces	Count (log ₁₀ per ml) of presumed pathogens in faeces
1	EEC 0125:B15	8.4	EEC 0125:B15	10.1
2	EEC 0125:B15	5.0	EEC 0125:B15	7.9
	EEC 0111:B4	4.5	EEC 0111:B4	9.0
	<i>E. coli</i>	4.4		
3	EEC 026:B6	5.2	EEC 026:B6	7.4
	<i>E. coli</i>	4.2		
4	EEC 0119:B14	4.2	EEC 0119:B14	8.8
	<i>E. coli</i>	4.2	EEC 0111:B4	7.9
	<i>Klebsiella</i> sp.	4.8		
5	EEC 0128:B12	8.6	EEC 0128:B12	9.4
	<i>Pseudomonas</i> sp.	6.0		
6	<i>Vibrio eltor</i>	6.3	<i>Vibrio eltor</i>	5.0
	Ogawa		Ogawa	
7	<i>Klebsiella</i> sp.*	3.5	<i>Klebsiella</i> sp.	6.0
8	<i>Pseudomonas</i> sp.*	7.2	<i>Pseudomonas</i> sp.	8.3
9	<i>E. coli</i>	4.5	<i>Salmonella</i> sp. (group B)	L
10	<i>E. coli</i>	3.0	<i>Shigella sonnei</i>	L
	<i>Pseudomonas</i> sp.	4.3		
11	<i>Klebsiella</i> sp.	4.6	EEC 0125:B15	6.6
12	<i>E. coli</i>	5.0	None isolated	...
13	<i>E. coli</i>	6.6	None isolated	...
14	<i>E. coli</i>	3.2	None isolated	...
	<i>Klebsiella</i> sp.	4.1	None isolated	...
15	<i>E. coli</i>	3.4	None isolated	...
	<i>Klebsiella</i> sp.	2.6	None isolated	...
16	<i>Klebsiella</i> sp.	5.0	None isolated	...
	<i>E. coli</i>	5.8	None isolated	...
	NFR	4.7	None isolated	...
17	<i>Pseudomonas</i> sp.	3.5	None isolated	...
18	NFR	3.6	None isolated	...
19	None isolated	...	None isolated	...
20	None isolated	...	EEC 0111:B4	7.9
21	None isolated	...	EEC 026:B6	9.2
22	None isolated	...	EEC 0128:B12	9.3

* See text for discussion on pathogenicity.

L = Present in low concentration.

NFR = Non-fermenting aerobic Gram-negative rod other than *Pseudomonas* spp.

EEC = Enteropathogenic *E. coli*.

The only available study of the jejunal flora of infants with acute gastro-enteritis is that of Bishop *et al.* (1974a), who found a " faecal type flora " in the duodenum in one-third of the children and no Gram-negative aerobic rods in the duodenum of the remainder; this last group resembled our patients with rotaviruses. In adults, Koya, Kosakai and Fukasawa (1954) gave EEC by mouth to healthy volunteers and showed that in subjects who developed mild diarrhoea a mixed jejunal microflora occurred, consisting of the EEC strain administered

as well as other coliforms, while in those who developed severe diarrhoea the strain of EEC that had been administered predominated. Gorbach *et al.* (1971) also found that patients with a "mixed flora" in the upper small intestine had a relatively mild disease. The present study, however, failed to show a correlation between the severity of diarrhoea as judged by the degree of dehydration, and the relative prevalence of pathogens in the jejunal aspirate.

There is very little information on the distribution of pathogens in the intestine in acute gastroenteritis. Thomson (1955) demonstrated EEC in the upper small intestine of one child with diarrhoea and Challacombe *et al.* (1974b) recovered the same EEC serotype from the duodenum and rectum of a child with acute diarrhoea. Gorbach *et al.* (1970, 1971) demonstrated *Vibrio cholerae* in the upper small intestine and stools of children with cholera, and toxigenic *E. coli* in the jejunum and stools of adults with acute undifferentiated diarrhoea.

In the present study, where children had the same pathogens in the stool and jejunal juice (patients 1-6) it may be assumed that these caused the diarrhoea. The jejunal aspirate of two infants (nos. 7 and 8) grew pure cultures of klebsiellae and pseudomonads respectively; since these were the only Gram-negative aerobic rods cultured they were presumed to be responsible for the disease. Apart from *E. coli*, other organisms have also been incriminated as a cause of gastroenteritis and diarrhoea; for example, klebsiellae in infants (Olarie *et al.*, 1961) and in patients with tropical sprue (Klipstein *et al.*, 1973) and pseudomonads in infants (Henderson, Maclaurin and Scott, 1969; Wadstrom *et al.*, 1976; Ferrao and Mavinkurve, 1977). These organisms were present in the jejunum in several of the infants, either alone or with other organisms, and in certain instances may have caused the illness.

When rotaviruses were present they presumably caused the disease (Bishop *et al.*, 1947b; Flewett, Davies, Bryden and Robertson, 1974; Middleton *et al.*, 1974). In one patient, EEC was also present, but as it was not found in the jejunum it was probably coincidental. Only one child with rotavirus had Gram-negative aerobic rods in the jejunal juice. This child is in striking contrast with patients 1-18 and the findings indicate that the symptoms must have been due to the virus and not to secondary bacterial overgrowth of the upper small intestine.

Three of the four children (patients 19-22) with diarrhoea not associated with rotavirus and without Gram-negative aerobic rods in the jejunum had EEC in their stools; the EEC, however, may not have caused the illness as they were not found in the upper jejunum.

In the three patients (nos. 9-11) in whom a pathogen occurred in the stool but not in the jejunal juice, it is difficult to be sure of the aetiology of the disease. In the two children with *Salmonella* and *Shigella*, the absence of the organisms from the jejunal juice was not unexpected, as colonisation occurs in the terminal ileum and colon. Shigellosis usually presents as a dysentery-like illness rather than as gastroenteritis and it seems improbable that it was the cause of the illness. The *Salmonella* may or may not have been the cause of the diarrhoea.

In the third child, the absence of EEC in the jejunum makes it likely that the organism was incidental to, rather than the cause of, the illness.

The cause of gastroenteritis in patients 12-18 is undetermined. Typing sera were available for only 10 EEC serotypes. Had more sera been available, other pathogenic serotypes of *E. coli* would probably have been found in both the jejunal samples and the stools of this group. Other *E. coli* serotypes may produce gastroenteritis (Sakazaki, Tamura and Saito, 1967).

The demonstration of enterotoxin has been considered important in deciding whether an organism is pathogenic (Guerrant *et al.*, 1975; Sack *et al.*, 1975). Gross, Scotland and Rowe (1976) found that traditional EEC serotypes, which had caused outbreaks of infantile diarrhoea, were negative for toxin production, and surmised that these serotypes produced diarrhoea by a hitherto unknown mechanism. However, evidence is accumulating that enterotoxin may be produced by serotypes (e.g., 06, 08, 015, 078 etc.) different from the classical ones (Ørskov *et al.*, 1976; Evans *et al.*, 1977). In the present study, isolates were not tested for toxin production and it is difficult to speculate on the pathogenetic mechanism.

The present studies suggest that when a potentially pathogenic bacterium is to be incriminated as a cause of gastroenteritis, its demonstration in the jejunum may be an important criterion in establishing its pathogenicity. It is recommended that future studies on the aetiology of acute gastroenteritis in childhood should always include studies of jejunal flora.

SUMMARY

The microbial flora of the jejunal lumen of 28 infants with acute gastroenteritis was compared with that of a group of 10 normal infants. The jejunum of control subjects harboured an "oral" type of flora and in a few instances enterobacteria in small numbers. The concentrations of all but one of the groups of organisms were higher in the patients than in controls, and the differences were of statistical significance for enterobacteria and lactobacilli. In eight subjects, the same pathogen was identified in the jejunum and the stool. In six subjects with rotavirus infection, there were almost no Gram-negative aerobic rods in the jejunum. The possible role of other Gram-negative aerobic rods in producing gastroenteritis is discussed. It is suggested that studies of jejunal flora are of considerable importance in assigning an aetiological role to bacteria in the causation of acute gastroenteritis.

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