The role of diarrhoeagenic *Escherichia coli* in acute diarrhoeal diseases in Bandar-Abbas, Iran

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Summary. The prevalence of different types of diarrhoea-producing *Escherichia coli* was measured in 273 patients attending 12 out-patient clinics in Bandar-Abbas, State of Hormozgan, Iran, during March 1984. Enteropathogenic *E. coli* (EPEC) belonging to 12 different serogroups, of which O128 and O126 were the most common, were found in almost 31% of the patients. Enterotoxigenic strains of *E. coli* (ETEC) were the next most frequent group (21.9%); among these, 36 (60%) strains produced heat-stable enterotoxin (ST), 14 (23.3%) strains produced both heat-labile enterotoxin (LT) and ST, and 10 (16.7%) strains produced LT only. The same pattern of toxigenicity was observed among the EPEC isolates. Ten of the 12 serogroups encountered in this study contained toxin producers, amongst which strains producing ST were dominant. Enteroinvasive *E. coli* (EIEC) strains were not isolated. These findings suggest that enterotoxin-producing *E. coli* may be an important cause of diarrhoea in this part of Iran.

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) have been reported to be a major cause of diarrhoea in man throughout the world (Sack, 1975; Rowe *et al.*, 1977), notably in developing countries (Merson *et al.*, 1980). They have also been found to be the commonest cause of travellers' diarrhoea in all countries where surveys have been conducted (Merson *et al.*, 1976; Echeverria *et al.*, 1981; Ryder *et al.*, 1981). The species causes diarrhoea by producing a heat-labile enterotoxin (LT) or a heat-stable enterotoxin (ST) or both. The ability to produce enterotoxin is usually plasmid mediated and easily lost from some strains, especially on subculture, probably due to loss of these plasmids (Evans *et al.*, 1977). Available studies suggest geographical differences in the types of enterotoxin produced by these organisms. For example, reports from India, in which cholera-like disease is endemic, consistently imply that strains producing LT are dominant (Ganguly *et al.*, 1980; Sen *et al.*, 1984), whereas in Bangladesh strains producing both LT and ST are most common (Sack *et al.*, 1977; Merson *et al.*, 1979). Nonetheless, it seems that all three enterotoxin types are found in all geographical areas.

The single report of the isolation of ETEC as a cause of infantile diarrhoea in Iran (Mohadjer *et*

al., 1982), indicates the presence of LT and ST-, and ST-producing strains, in Tehran. In view of the scarcity of data on ETEC as a cause of diarrhoea in Iran, this study was undertaken to establish the role of these pathogens in endemic diarrhoea in different parts of the country and describes, in part, the results of an investigation into the association of different types of enterotoxigenic *E. coli* with diarrhoea in Bandar-Abbas, State of Hormozgan, Iran.

Materials and methods

Collection of specimens

Faecal specimens were collected from 273 patients with diarrhoea attending 12 out-patient clinics in Bandar-Abbas, Hormozgan, during March 1984. Diarrhoea was defined as the passage of six or more unformed stools or four or more unformed stools with blood, per day. None of the patients had taken antibiotics in the preceding week. The specimens were collected on cotton swabs with charcoal and placed in modified Stuart's medium in screw-capped tubes (Gastrin *et al.*, 1968). These were stored and transported in an ice-box to the Pasteur Institute of Iran, Tehran, for microbiological examination.

Cultivation and isolation

Within one month of collection, the faecal specimens were cultured on the following media: MacConkey Agar

(Difco); Salmonella and Shigella Agar (Difco), and Thiosulfate-Citrate-Bile salts-Sucrose (TCBS) Agar (Difco). They were inoculated also into Selenite-F broth (BBL) and alkaline peptone water (Difco) and subcultured on to the solid media listed above after incubation at 37°C overnight. At least five lactose-fermenting colonies with the appearance of *E. coli* and two colonies of other potential pathogens were identified by the methods of Edwards and Ewing (1972). Identification of EPEC was by slide agglutination with polyvalent and monovalent OK antisera (Difco), and was confirmed by tube agglutination.

Testing for ETEC strains

A loopful of bacterial growth from nutrient agar slants was inoculated into 250 ml Erlenmeyer flasks containing 10 ml of Trypticase Soy Broth (Difco) supplemented with yeast extract 0.6%. The flasks were incubated at 37°C overnight on a shaker at 200 rpm. The cultures were centrifuged at 4000 rpm for 20 min at 4°C and supernates assayed for heat-labile toxin (LT) in adult rabbit ileal loops (De and Chatterjee, 1953). As a positive control, a culture filtrate of *E. coli* strain H-10407 (provided by Dr Orskov) containing a non-self-transmissible plasmid coding for LT and ST, was prepared and tested in the same way. Normal saline served as a negative control. A ratio of volume to length (ml/cm) ≥ 1 was taken to indicate the presence of LT. Samples of the same culture supernates were also tested for the presence of heat-stable toxin (ST) in 1-4-day-old suckling mice (Dean *et al.*, 1972). A mean ratio of intestinal weight to remaining carcass weight > 0.083 was taken to indicate the presence of ST.

Enteropathogenic *E. coli* (EPEC) were also tested for toxin production. They were grown on trypticase soy agar slants containing yeast extract 0.6% and lincomycin 90 µg/ml and incubated at 37°C overnight. Cultures were then washed with 3 ml of normal saline containing polymixin B 10 000 units/ml and shaken at 100 rpm for 30 min at 37°C. These were centrifuged at 4000 rpm for 15-20 min and supernates were assayed for the presence of LT in the adult rabbit ileal loop and by an agglutination-inhibition method with VET-RPLA Kits (Denka Seiken Co., Japan). These strains were also grown in Erlenmeyer flasks containing trypticase soy broth with yeast extract and tested for ST production as described above.

Testing for EIEC strains

All lactose-fermenting and 28 lactose non-fermenting isolates of *E. coli* were tested for their ability to cause keratoconjunctivitis in the guinea-pig eye (Sereny, 1955).

Results

ETEC were detected in the faeces of 60 patients (21.9%), aged 3 months to 48 years. In contrast, EPEC were isolated from the faeces of 84 patients

(30.7%) <3 years old. Salmonellae were isolated from 14 patients (5.1%) and shigellae from 4 (1.5%). EIEC, *Vibrio cholerae* and *V. parahaemolyticus* were not isolated during the course of this study. Of the 60 ETEC isolates, 36 (60%) produced ST, 10 (16.6%) produced LT and 14 (23.3%) produced both LT and ST (table I). Ten patients (3.6%) with mixed infections were encountered: ETEC and a salmonella in 4, and one patient had ETEC and a shigella. The same pattern of mixed infection was also seen with the EPEC strains.

The 84 EPEC strains isolated belonged to 12 different serogroups, of which O128:K67 (22.6%) and O126:K71 (21.4%) comprised 44% of the isolates. Seventy-seven of 84 EPEC strains, belonging to 10 of the 12 serogroups, were tested for toxin production; 26 (33.7%) produced ST or LT or both (table II). Strains producing ST were the most

Table I. Distribution of EPEC and ETEC in relation to age among 273 patients

Age group (years)	Number of patients investigated	Number of <i>E. coli</i> isolated			
		EPEC	ETEC		
			LT	ST	LT/ST
<1	103	54	5	11	5
1-2	63	22	3	7	5
2-3	23	8	1	3	0
3-4	10	0	0	2	1
4-5	13	0	0	2	1
>5	61	0	1	11	2
Total	273	84	10	36	14

Table II. Frequency and type of toxin produced by EPEC serogroups isolated from 273 patients

EPEC serogroups	Number of isolates (Number tested)	Percentage of total	Toxin type		
			LT	ST	LT+ST
O20ab:K84	2 (2)	2.38	0	1	0
O26:K60	5 (2)	5.95	0	1	0
O44:K74	1 (1)	1.19	0	0	0
O55:K59	4 (4)	4.76	1	1	0
O86a:K61	7 (6)	8.33	0	2	1
O111:K58	7 (7)	8.33	0	3	0
O119:K69	5 (5)	5.95	0	1	0
O124:K72	2 (1)	2.38	0	0	0
O125:K70	7 (7)	8.33	0	1	0
O126:K71	18 (18)	21.43	3	3	0
O127a:K63	7 (7)	8.33	1	1	1
O128:K69	19 (17)	22.62	0	3	2
Total	84 (77)	100	5	17	4

frequent, comprising >65% of the enterotoxigenic strains of EPEC. Strains producing both LT and ST were the least common and only 5 (6.4%) of EPEC isolates tested produced LT.

Discussion

Many reports indicate that ETEC are amongst the commonest causes of intestinal disease in different parts of the world (e.g., Nalin *et al.*, 1975; Sack, 1975; Rowe *et al.*, 1977). The importance of this pathogen in diarrhoeal disease in Iran has been little studied, probably because the complex biological assays needed to detect enterotoxins make them impractical in clinical laboratories and field studies. The present study demonstrates an association of all three types of ETEC with acute diarrhoea in the temperate state of Hormozgan in south Iran; >21% of the patients studied who were between the ages of 3 months and 48 years were infected with ETEC. Strains producing ST were commonest followed by those producing both LT and ST. These results are similar to several reported from elsewhere (Sack *et al.*, 1975; Evans *et al.*, 1977), but differ from those obtained in Bangladesh (Ryder *et al.*, 1976; Sack *et al.*, 1977; Merson *et al.*, 1979) where strains producing LT and ST predominate, and in India (Sen *et al.*, 1984) and other parts of the world (Guerrant *et al.*, 1975) where strains producing LT have been more frequently found. This suggests that strains producing ST and those producing both LT and ST are important causes of diarrhoea in patients of all ages in Bandar-Abbas. It must be noted that the month's interval between collection and culturing of the stool samples might

have led to an underestimation of the prevalence of such isolates.

On the other hand, EPEC strains seem to be more important in the aetiology of infantile diarrhoea only. The association of certain OK serogroups, known as EPEC, with infantile diarrhoea has been established in many parts of the world (Bhagwan *et al.*, 1975; Gurwith *et al.*, 1978), even though the mechanism by which they cause the disease has not been clearly defined. The possibility that these strains of human origin produce enterotoxin has been investigated (Smith and Gyles, 1970; Reis *et al.*, 1979). Despite many investigations of different serogroups of EPEC isolated from patients with epidemic or sporadic diarrhoea, few strains have been shown to produce toxins (Gurwith *et al.*, 1978; Levine *et al.*, 1978). Temporary acquisition of an enterotoxin plasmid, or incomplete characterisation of EPEC serogroups, have been given as explanations for these findings (Robins-Browne, 1987). The results of the present study show the association of different EPEC serogroups with endemic diarrhoea in children under 3 years of age in Bandar-Abbas, and also that at least 33% of these strains produce LT, ST or both toxins. EPEC strains producing ST were the commonest. In view of their predominance among the ETEC isolates this is not surprising, as transfer of enterotoxin plasmids from ETEC to other bacteria by conjugation is common (Smith and Halls, 1968; Gyles *et al.*, 1974).

Further studies now in progress may yield a better understanding of the importance of *E. coli* in diarrhoea in children and adults in other parts of Iran.

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