JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 630–633
0095-1137/85/040630-04$02.00/0

Vol. 21, No. 4

Survey in Vanuatu on Enterotoxigenic *Escherichia coli* in Children and Infants With and Without Acute Diarrhea

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Received 1 October 1984/Accepted 7 January 1985

We have studied the incidence of enterotoxigenic *Escherichia coli* (ETEC) strains isolated from infants with and without diarrheal diseases in Vanuatu, South Pacific. Over a period of 5 months we have isolated enterotoxigenic *E. coli* strains from 29 (26.6%) of 109 children with acute diarrhea and from 13 (21.6%) of 60 children of the control group. In the group with diarrhea, 7 (6.4%) strains released heat-labile toxin, 7 (6.4%) released heat-stable toxin, and 15 (13.7%) produced both heat-labile and heat-stable toxin. In the control group, only one strain (1.6%) produced heat-stable toxin, 12 (20%) produced heat-labile toxin, and none produced both. Association of strains releasing heat-stable toxin or both heat-labile and heat-stable toxin with diarrhea was highly significant as shown by statistical analysis. The O serogroups and colonization factors CFA/I and CFA/II are presented.

Diarrheal disease is a significant health problem in developed countries (39) and a major problem in developing countries (20, 39). Enterotoxigenic *Escherichia coli* (ETEC) is now recognized as a cause of gastroenteritis in animals and especially in infants and young children (12, 39). Many reports have been published on ETEC stains (1, 15, 31, 34, 39), enterotoxins (6, 7, 18, 21, 24, 27), colonization factors (8, 13, 15, 23), and their plasmids (13, 14, 15, 35). The recent status of knowledge about *E. coli* diarrhea was adequately revised by the World Health Organization (39, 40). However, the epidemiology of ETEC disease in some geographical areas must be investigated. Most studies carried out so far indicate that ETEC strains are not numerically important as a cause of endemic infantile diarrhea in developed countries (10, 29). However, in the South Pacific, as in some developing countries, the role played by ETEC strains in endemic diarrhea in children is not clear. According to the results of some studies, ETEC strains represent a major cause of diarrhea, whereas in other studies they have been found at a low frequency (19, 29, 39), and the rate of isolation has been the same in sick patients and controls (9). In 1979 in the South Pacific, symptoms of gastroenteritis were reported in 26,314 individuals (28). In some areas, cases at a rate of 3,800 per 10,000 population are reported in infants annually (36).

This work was undertaken to find the serogroups, the enterotoxigenicity, and the colonization factor antigens (CFAs) of the strains of *E. coli* isolated from infants with acute diarrhea and to study the frequency of ETEC strains in children with and without diarrhea in Vanuatu, a country in the South Pacific.

MATERIALS AND METHODS

Children surveyed. Stool specimens were taken from 169 children under 10 years of age who were hospitalized in the pediatric section of the hospital at Port Vila or were attending the dispensaries near Port Vila during the period February to July 1984. Of these children, 109 were affected by diarrhea (patients) and 60 were without gastrointestinal illness (controls). The children had received no antibiotics for at least 2 months preceding sampling. The children in the two groups were of approximately the same ages and the same socioeconomic level.

Bacteriological methods. Feces were collected after natural evacuation, immediately inoculated into TGV medium (Institut Pasteur Production), and transported by air to the Pasteur Institute in Nouméa for testing. All the fecal specimens were examined by standard procedures for *Shigella*, *Salmonella*, *Vibrio*, and enteropathogenic *E. coli* (EPEC) serogroups by the methods recommended by Le Minor and Veron (22).

Isolation and identification of ETEC strains. Five representative strains of *E. coli* were selected at random and studied. They showed morphological, cultural, and biochemical characteristics conforming to the pattern for *E. coli* laid down by Le Minor and Veron (22). After selection, strains were stored in maintenance medium (5) for 1 week before toxin testing, serogrouping, and identification of colonization factors were done.

Enterotoxin testing. The strains were tested for the production of heat-labile toxin (LT) by the ganglioside immunosorbent assay (37) and for the production of heat-stable toxin (ST) by the suckling mouse assay (17). The strains were assayed on Vero cells (Flow Laboratories) for the presence of Vero toxin (VT) (21). The Vero cell line was tested for VT sensitivity with the *E. coli* H19 (026:H11) VT kindly provided by B. Rowe (Central Public Health Laboratory, Division of Enteric Pathogens, London, England) as the control strain.

Serotyping. All strains were serotyped in the Pasteur Institute with *E. coli* O antisera (Difco, Hoechst-Behring, and Institut Pasteur Production) by previously described methods (11).

Detection of CFA/I and CFA/II. Detection of CFA/I and CFA/II was performed by using rabbit-specific antisera obtained and adsorbed by the method described previously (12, 14, 31) and by mannose-resistant hemagglutination with human and bovine erythrocytes (13). Before testing, the strains were cultivated on CFA agar as described by Evans et al. (13).

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TABLE 1. Frequency of ETEC in 109 children with diarrhea and 60 healthy children from Vanuatu

ETEC strain	No. (%) of strains in:	
	Sick children	Control group
LT	7 (6.4)	12 (20)
ST	7 (6.4)	1 (1.6)
LT/ST	15 (13.7)	0 (0)

RESULTS

Tests for enterotoxigenicity showed that 29 ETEC strains (26.6%) were isolated from 109 children and 13 ETEC strains (21.6%) were isolated from the control group of 60 children. From the five random *E. coli* isolates from each patient, ETEC strains were recorded with the following frequencies: experimental group, 4 ETEC strains, twice; 3 ETEC strains, 8 times; 2 ETEC strains, twice; 1 ETEC strain, 17 times; control group, 4 ETEC strains, twice; 3 ETEC strains, 8 times; 2 ETEC strains, twice; 1 ETEC strain, once. Strains producing LT were found in 7 children with diarrhea (6.4%) and 12 controls (20%). Strains producing ST were found in seven sick children (6.4%) and one control (1.6%). Strains producing both LT and ST were found in 15 sick children (13.7%) and no controls (Table 1). The strains assayed on Vero cells did not produce Vero cytotoxin. Statistical analysis by the Fisher exact test showed that both ST and LT/ST strains were associated with diarrhea ($P = 0.0165$). However, the frequency of LT strains was higher in the control group, as shown by the chi-square test ($\chi^2 = 24.6$; $\alpha < 0.001$).

Serogrouping showed that 37 (21.8%) strains were untypable and 17 (10%) were rough. The LT strains isolated from children with diarrhea belong to the O6, O8, and O63 serogroups, and the LT strains isolated from the control group belong to the O25 and O115 serogroups. The ST strain isolated from the control group belongs to the O78 serogroup. Of the strains isolated from the group with diarrhea, one ST strain belongs to O25 and three ST strains each belong to O57 and O78. Three of the ST/LT strains belong to O6, one belongs to O24, two belong to O25, one belongs to O40, and four each belong to O75 and O78.

Results of correlation between O serogroups and the enterotoxigenicity of *E. coli* in the group with diarrhea revealed that strains belonging to O6, O25, and O78 were 100% toxigenic.

Cases in which the O57, O75, and O78 serogroups were harbored were distributed throughout the period of the study but all came from the pediatric section of the hospital.

We have identified nine EPEC isolates from nine different patients. Six patients harbored the O26:B6 serogroup, and the remaining three harbored only one of the O126:B16, O111:B4, or O55:B5 serogroups. Such EPEC strains were not found in the controls. None of the EPEC strains was capable of releasing LT, ST, or VT, and the Vero cell line was VT sensitive to the control strain H19.

The colonization factors CFA/I and CFA/II were only identified in the group with diarrhea in seven strains releasing ST or both ST and LT (30%). None of the strains isolated from the control group or the LT strains had CFA/I or CFA/II. Four ETEC strains produced CFA/I; two of these were ST strains of serogroup O78 (28.6%), and two were ST/LT strains of serogroup O25 (13.3%). CFA/II was detected only in the three ST/LT strains of serogroup O6. The four strains CFA/I cause mannose-resistant hemagglutination of human and bovine erythrocytes, and the three strains CFA/II cause mannose-resistant hemagglutination of bovine erythrocytes only.

In the group with diarrhea, three LT strains were associated with a *Shigella* strain, a *Salmonella* strain, and an EPEC strain belonging to 0126:B16 serogroup. A *Salmonella* strain was also isolated in association with an LT/ST strain.

DISCUSSION

Results obtained in this study suggest that infant diarrheal disease in Vanuatu is frequently caused by strains of ETEC. However, the frequency of isolation of ETEC strains as a whole was almost the same in sick children and in controls. This was because ETEC strains releasing LT were isolated at a higher frequency from the group without diarrhea. This result suggests that *E. coli* strains releasing LT were not an important etiological agent in diarrhea in this study. On the other hand, ETEC strains releasing LT were isolated in sick patients in association with classic enteropathogenic bacteria.

Similar isolation rates of ETEC strains in children were reported in studies of endemic diarrhea involving infants with diarrhea and control groups (9, 19, 30). In all the communications, as well as in our study, no ST/LT strains were isolated from children without diarrhea, and ETEC strains releasing ST were found less frequently than LT strains in control groups.

Our results of enterotoxigenicity of the strains isolated in this survey are not in agreement with those reported from different studies in this region of the South Pacific. The preponderance among ETEC strains of ST- or LT/ST-producing strains over LT-producing strains had not been noted in a group of infants with diarrhea in other islands in the South Pacific (16).

The frequency of ETEC strains in sick infants in this study is higher than that reported in developed countries (29, 39) but is similar to that reported in Brazil (19, 21, 30) and significantly lower than that reported in Bangladesh (20). However, the sensitivity of our diagnostic procedure for ETEC would be expected to be somewhat low, because only five colonies of *E. coli* were tested per patient.

In our region of the South Pacific, several surveys have been carried out and discrepancies have been found (4, 16). They seemed to be related to the time interval of the studies and the socioeconomic level of the population.

No strains from the 109 strains assayed on Vero cells (including enteropathogenic serogroups) were positive for VT production, in contrast to results obtained by Wade et al. (38), who isolated 5.4% of VT-positive strains belonging to the enteropathogenic O26 serogroup. Furthermore, none of the EPEC strains isolated released LT or ST, as suggested by many communications on this topic. Recent studies suggest that EPEC strains cause diarrhea by other mechanisms; Robins-Browne et al. (32) failed to show conventional enterotoxin synthesis by classical EPEC strains of proven pathogenicity.

A large proportion of our strains were either untypable or rough. This has been the experience of other workers (2, 20), who obtained 36% untypable and 16% rough strains. Some of our typable strains of *E. coli* showed similar serotypes to those reported elsewhere in New Zealand (4), Australia (3), and New Caledonia (16) but differed somewhat from those found by Merson et al. (25). Although only the strains belonging to serogroups O6 and O78 were isolated in conformation with their general character, those belonging to serogroups O25 and O78, two of the most commonly encoun-

tered serotypes in infantile diarrhea (25, 39) were also isolated in our study. They had been considered particularly common enterotoxigenic strains, regardless of geographical location (33, 39). However, as far as we could determine, no communication from this location of the South Pacific has yet shown the presence of the serogroup O25.

Strains belonging to serogroups O57 and O75, of which seven were isolated, have not been included in the list of ETEC (25) except in India (2).

In addition to these, strains belonging to serogroups O24 and O40 were also enterotoxigenic. This opens up a possibility that enterotoxigenic serogroups in this area are probably different from the serotypes proposed by authors in exhaustive works (25).

Our ETEC strains possessed O24, O40, O57, and O75 antigens which have never been associated with toxin production (25, 26, 31, 33, 39). This suggests that the combinations used in the polyvalent antisera proposed by Merson et al. (26) are not likely to detect LT+ or ST+ ETEC strains from infants in the area studied. In the group with diarrhea, only 68% (20 of 29) of our ETEC strains would have been detected by their proposed method.

The distribution of CFA among O serotypes was similar to that reported by others (13, 31), but the association of CFA with ETEC strains producing ST or both ST and LT suggest that single plasmids may code for both ST and CFA/I (12, 14, 15).

All these results seem to indicate that other studies must be done to understand the exact role played by ETEC toxin phenotypes in infant endemic diarrhea, especially with regard to ETEC strains releasing LT.

ACKNOWLEDGMENTS

We thank the Authorities of the National Health Office in Vanuatu and the French Ambassy in Port Vila for facilities and assistance. The cooperation of R. Taylor, Epidemiologist of the South Pacific Commission, is also appreciated. We are indebted to E. Begaud, A. Rivaton, J. F. Marin, B. Batson, and A. Owen for technical assistance and to D. Kalorib for his assistance.

This work is supported by a joint grant from the South Pacific Commission (grant reference, SPC 3/1, folio 1/83).

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