JOURNAL OF CLINICAL MICROBIOLOGY, June 1982, p. 1062–1064
0095-1137/82/061062-03$02.00/0

Vol. 15, No. 6

# Frequency of *Escherichia coli* Strains Producing Heat-Labile Toxin or Heat-Stable Toxin or Both in Children With and Without Diarrhea in São Paulo

M. HENRIQUETA L. REIS,[1]* BEATRIZ E. C. GUTH,[1] TANIA A. T. GOMES,[1] JAYME MURAHOVSCHI,[2] AND LUIZ R. TRABULSI[1]

*Department of Microbiology, Immunology, and Parasitology, Escola Paulista de Medicine,[1] and Clínica Infantil do Ipiranga,[2] São Paulo, SP, Brazil*

Received 21 October 1981/Accepted 22 February 1982

Enterotoxigenic *Escherichia coli* strains were isolated from 32 (13.4%) of 245 children with diarrhea and from 11 (11.4%) of 96 children of the control group. Strains producing heat-labile toxin were found more frequently in normal children than in children with diarrhea. Strains producing heat-stable toxin and both heat-labile and heat-stable toxins were isolated only from children with diarrhea. Association of these strains with diarrhea was highly significant as shown by statistical analysis. The O:H types and the colonization factors of strains producing heat-stable toxin and both heat-labile and heat-stable toxins are presented.

Enterotoxigenic *Escherichia coli* (ETEC) may produce heat-labile toxin (LT), heat-stable toxin (ST), or both (LT/ST). Most studies carried out so far indicate that these bacteria are not numerically important as a cause of endemic infantile diarrhea in developed countries (6, 13, 16). In developing countries the role played by ETEC strains in endemic diarrhea of children is not clear. According to the results of some studies, they represent a major cause of diarrhea, whereas in other studies they have been found at a low frequency (3, 10, 12, 16). On the other hand, in some studies the rate of isolation has been the same in patients and controls or even higher in controls (5, 19). The purpose of this paper is to report the results of a 2-year study of the frequency of LT, ST, and LT/ST *E. coli* strains in children with and without endemic diarrhea in São Paulo, Brazil.

## MATERIALS AND METHODS

**Population studied.** A total of 341 children attending two outpatient clinics in São Paulo were studied for the presence of ETEC strains in their feces. Of these children, 245 had fluid diarrheal stools when they visited the clinic. The illness was present for at least 24 h but for no longer than 5 days before evaluation. Ninety-six of the children had no symptoms of gastrointestinal disorders at the time of the visit. Children of the two groups were of approximately the same ages (95% of them were less than 2 years old), were of the same socioeconomic level, and had not received any antimicrobial agents at least in the preceding week. The study was conducted between February 1978 and December 1979, but around 80% of the cases were studied during the summer months.

**Isolation and identification of ETEC.** Feces were collected after natural evacuation. When necessary, evacuation was stimulated with a glycerol suppository. Feces were plated between 1 and 3 h after collection. When the feces could not reach the laboratory within this time, they were kept in buffered glycerol-saline solution (7) at room temperature until the next day. However, this solution was used in fewer than 5% of the cases.

LT and ST were assayed in 5 to 10 *E. coli* colonies grown on MacConkey agar plates. The Y1 adrenal cell culture assay was used for LT (2), and the infant mouse assay was used for ST (1). For the assay of both toxins, strains were cultivated by aeration in CYE medium (9). LT was assayed within 1 week of isolation of the *E. coli* strains. Supernatants for the assay of ST were collected at the same time, frozen at −20°C, and assayed within 2 months. Appropriate controls were included in all assays for both enterotoxins.

The O and H antigens were determined by agglutination tests as recommended by Edwards and Ewing (7). Colonization factor antigens I and II (CFA/I and CFA/II) were detected by mannose-resistant hemagglutination with human and bovine erythrocytes (8). Their presence was confirmed by slide agglutination tests with specific CFA/I and CFA/II antisera. These antisera were prepared in our laboratory, as previously described (11), or provided by D. G. Evans, University of Texas Health Science Center, Houston. Feces of all children were also cultured for *Shigella*, *Salmonella*, and enteropathogenic *E. coli* serotypes, according to the methods recommended by Edwards and Ewing (7).

## RESULTS

ETEC strains were isolated from 32 (13.4%) of the children with diarrhea and from 11 (11.4%) of the children in the control group. LT strains

TABLE 1. Frequency of LT, ST, and LT/ST *E. coli* strains in children with and without diarrhea in São Paulo

| Strains | Frequency | | | |
|---|---|---|---|---|
| | Diarrhea group (245 cases) | | Control group (96 cases) | |
| | No. | % | No. | % |
| LT | 8 | 3.3 | 11 | 11.4 |
| ST | 12 | 4.9 | 0 | 0 |
| LT/ST | 12 | 4.9 | 0 | 0 |

were found in 8 (3.3%) ill children and in 11 (11.4%) controls; ST and LT/ST strains were each found in 12 ill children and in no controls (Table 1).

Statistical analysis by the Fisher exact test showed that both ST and LT/ST strains were associated with diarrhea ($P = 0.0175$). However, the frequency of LT strains was higher in children without diarrhea, as shown by the $\chi^2$ test ($P < 0.01$).

The CFA/I and CFA/II were detected in 10 (45%) of the 24 ST and LT/ST strains. None of the LT strains had CFA/I or CFA/II. CFA/I was produced by four (33%) of the ST strains and by three (25%) of the LT/ST strains. CFA/II was detected only in the LT/ST strains (25% of the strains) (Table 2).

The O:H types of the ST and LT/ST strains and their respective colonization factors are given in Table 3. LT strains belonged to several O groups (18), in general, not related to the O:H types given in Table 3. LT strain serotypes isolated from children with diarrhea were different from those isolated from children in the control group, with two exceptions. In serogroup O128ac, only serotype O128ac:H12 produced a colonization factor (CFA/I). This factor was also found in serotype O63:H$^-$. CFA/II was detected only in serotype O6:H16 (Table 3). Cases harboring the main O:H types were distributed throughout the period of the study and came from different neighborhoods. LT strains were associated with *Shigella* in two normal children and with *Shigella* and *Salmonella* in three children with diarrhea. In one case harboring an ST strain, a *Salmonella* strain was also isolated, and one case harboring an LT/ST strain was associated with *E. coli* O111.

TABLE 2. Distribution of colonization factors in LT, ST, and LT/ST *E. coli* strains

| Strains | No. of strains | No. of strains with CFA | Type of CFA |
|---|---|---|---|
| LT | 19 | 0 | |
| ST | 12 | 4 | CFA/I |
| LT/ST | 12 | 6 | CFA/I (3)[a]<br>CFA/II (3)[a] |

[a] Number of strains.

TABLE 3. O:H types and colonization factors in ST and LT/ST *E. coli* strains isolated from children with diarrhea

| O:H type[a] | Total no. of strains isolated | Toxin type | No. of strains with CFA | Type of CFA |
|---|---|---|---|---|
| O128ac:H12 | 4 | ST | 4 | CFA/I |
| O128ac:H21 | 2 | ST | 0 | |
| O128ac:H27 | 1 | ST | 0 | |
| O128ac:H$^-$ | 1 | ST | 0 | |
| ND | 4 | ST | 0 | |
| O6:H16 | 5 | LT/ST | 3 | CFA/II |
| O25:H42 | 1 | LT/ST | 0 | |
| O63:H$^-$ | 3 | LT/ST | 3 | CFA/I |
| O139:H28 | 2 | LT/ST | 0 | |
| NT:H$^-$ | 1 | LT/ST | 0 | |

[a] NT, Negative O1 to O157; H$^-$, nonmotile; ND, not determined.

## DISCUSSION

The frequency of ETEC as a whole was the same in the children with diarrhea and in the control group. However, this was basically because LT strains were isolated at a higher frequency from the control group. At present we have no explanation for the high rate of isolation of LT strains from children without diarrhea, but this finding suggests that these strains were not an important etiological agent in diarrhea in this study population. A review of the studies of endemic diarrhea involving ill children and control groups and in which *E. coli* isolates were simultaneously tested for the production of LT and ST shows some similarities to and differences from this study. Guerrant et al. (12) found that among 38 children with diarrhea in Florianopolis, Brazil, 17 (44.7%) were carriers of LT strains, 3 (7.9%) were carriers of LT/ST strains, and 1 (2.6%) was a carrier of ST strains. Of 18 controls without diarrhea, the only ETEC strain isolated (5.5%) produced ST. In a study in the Philippines involving 82 children with diarrhea and 49 healthy controls, Echeverria et al. (4) obtained the following results. Six of the patients (7.3%) were carriers of LT/ST strains (three of these patients also had LT strains that belonged to the same serotypes as did LT/ST ones), three (3.6%) were carriers of ST strains, and none were carriers of LT strains. Among the control group, two (4.1%) carried LT strains, two carried ST strains, and none carried LT/ST strains. Pickering et al. (16) studied 595 children with diarrhea and 210 children without diarrhea in Houston and Mexico. They verified that LT

strains were present in 13 (2.2%) children with diarrhea and in 5 (2.4%) normal children; ST strains were isolated from 14 (2.3%) children with diarrhea and from 1 (0.5%) normal child. LT/ST strains were found in seven (1.2%) children with diarrhea and in none of the children in the control group. More recently, Magalhães et al. (15) found that of 60 children with diarrhea in Recife, Brazil, 7 (12%) were carriers of ETEC strains: 4 LT, 2 LT/ST, and 1 ST. No ETEC strains were isolated from 21 children without diarrhea. It is interesting that in all of the studies reviewed, as well as in this study, no LT/ST strains were isolated from children without diarrhea, and ST strains were found less frequently than LT strains in controls. These data show that more studies are necessary for a better understanding of the role played by ETEC toxin phenotypes in pediatric endemic diarrhea. This is particularly true with regard to LT strains.

The frequency of ETEC strains in children with diarrhea in this study was similar to that reported by Magalhães et al. in Recife (15) and significantly lower than that reported by Guerrant et al. in Florianopolis (12). In Mexico, where several studies have been carried out, such discrepancies have also been found (1, 5, 10). In some studies the discrepancies seemed to be related to the time interval of the studies and the socioeconomic level of the populations. Similar factors may have accounted for the different results of the studies conducted in Brazil.

The distribution of colonization factors among the O:H types of ETEC was similar to that reported by others (8, 18). The association of these factors with ST and LT/ST strains is interesting in view of the finding that single plasmids may code for both ST and CFA/I (17).

## ACKNOWLEDGMENTS

We are grateful to Neil Ferreira Novo for statistical analysis of the data.

This work was supported by a joint grant from the National Science Foundation and the Brazilian National Research Council under the Cooperative Science Program for Latin America and by a grant from Financiadora de Estudos e Projetos.

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