JOURNAL OF CLINICAL MICROBIOLOGY, July 1995, p. 1961–1962 Vol. 33, No. 7
0095-1137/95/$04.00+0

Retrospective Case-Control Study of Diffusely Adhering *Escherichia coli* and Clinical Features in Children with Diarrhea

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Received 28 December 1994/Returned for modification 15 March 1995/Accepted 13 April 1995

A retrospective case-control study with a small population group revealed that, among clinical signs, vomiting but not diarrhea was significantly associated with the presence of diffusely adhering *Escherichia coli* (DAEC) in children suffering from gastroenteritidis ($P < 0.05$). Of the children carrying DAEC strains, those who were F1845 DNA probe positive had a significantly longer hospital stay than those who were F1845 DNA probe negative. We believe that the heterogeneity of DAEC strains is responsible for the discrepant results concerning their involvement in disease and that only some of these strains are really pathogenic for children.

Although recent epidemiological studies have shown a high prevalence of diffusely adhering *Escherichia coli* (DAEC) strains among strains isolated from diarrheal stools (7, 8), their involvement is still a matter of debate. Their pathogenic nature has not been demonstrated in challenge studies with adult volunteers (11), and discrepant results concerning their association with diarrhea have been obtained. While a greater percentage of DAEC was isolated from diarrheal stools from children in Mexico and Bangladesh than from controls (1, 4, 6, 10), no significant association was found in studies performed in Chile, Brazil, and Thailand (2, 3, 5, 9).

One of the possible reasons for these differences is the criteria used to assign a strain to the DAEC category. Most studies used a pattern of adherence to either HEp-2 or HeLa cells as a classification method (1, 2, 5, 6, 10). However, when both adhesion assays and hybridization with specific DNA probes are performed, not all the *E. coli* strains adhering to cells in culture are detected by the hybridization procedure (4), suggesting that the DAEC group is heterogeneous.

In this study, we compared the clinical signs of a small group of children from whom only DAEC strains were isolated with those of patients without any potential enteropathogen. All the children selected were hospitalized in the gastroenterology unit of the pediatric ward of a hospital in Clermont-Ferrand, France. The DAEC strains isolated from the stools were previously characterized by pattern of adherence to HEp-2 cells and DNA hybridization with the *E. coli* F1845 *daaC* probe (8). The main aim of this study was to determine if the presence of DAEC (*daaC*-positive and *daaC*-negative) strains in children's stools was significantly associated with clinical features and thereby to assess the clinical impact of DAEC strains.

The subjects were children between the ages of 1 month and 14 years hospitalized for digestive disorders between 1989 and 1992. An initial selection of 39 individuals whose stools contained no recognized bacterial, viral, or protozoal pathogens was made. A DAEC strain was isolated by the HEp-2 adherence assay as previously described (8) from 24 of these children, who were included in the study as case patients, while the remaining 15 with DAEC-negative isolates were used as controls. Of the 24 DAEC-positive isolates, 13 hybridized with the *daaC* DNA probe.

The interviewing and specimen collection team consisted of trained medical personnel from Clermont-Ferrand hospitals. Diarrhea was defined as three or more abnormally loose stools per day for at least 3 days. The presence of blood in stools was determined by macroscopical examination. An episode was considered persistent if it continued for ≥14 days. Age, symptoms, and clinical signs were recorded on charts. Fever was defined as an oral temperature of >37.8°C (>100°F). Dehydration was defined by clinical characteristics, e.g., tacky mucous membranes, sunken eyes or fontanelle, and poor skin turgor. The percentage of dehydration was obtained by comparing body weights before and after the diarrheal episode. Additional demographic, clinical, and exposure information was obtained. Communities with fewer than 2,000 inhabitants were considered rural. Dietary habits during the 2 weeks before hospitalization, such as a lactose-free and/or cow milk protein-free and/or gluten-free and/or low-residue diet, were recorded.

Statistical analysis was done by the *t* test, the χ^2 test, or Fisher's exact test, with Epi-Info, version 5, software. The odds ratios were used to estimate the relative risks, and the 95% confidence interval was determined as described by Robins (10a).

A total of 37% of the DAEC strains were isolated from stools from children older than 18 months, and 46% of the DAEC-positive patients were female (Table 1). Although higher than those for controls (children without DAEC strains), these numbers were not significant ($P = 0.4$). No difference was noticed when environments were compared: 37% of the case patients lived in rural areas versus 47% of the controls. Four of the children carrying DAEC strains suffered from previous digestive diseases and were on a therapeutic diet. Two children with a lactose-free diet were among the DAEC carriers.

The comparison of clinical signs in children carrying DAEC and those in controls showed that the presence of DAEC was significantly associated with vomiting ($P < 0.05$) (Table 1).

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TABLE 1. General data and clinical signs associated with children carrying DAEC

Characteristic	No. of children (%)		RR[a]	CI[b]	*P*
	Patients (n = 24)	Controls (n = 15)			
Age, >18 months	9 (37)	3 (20)	1.9	0.6–5.8	0.4
Sex, female	11 (46)	4 (27)	1.7	0.7–4.4	0.4
Rural	9 (37)	7 (47)	1.9	0.6–5.8	0.3
Diet	6 (25)	0	ND[c]	ND	0.06
Previous digestive disease	4 (17)	0	ND	ND	0.1
Diarrhea					
≥3 stools/day	14 (61)	7 (47)	1.3	0.7–2.4	0.5
>4 stools/day	7 (30)	1 (7)	4.4	0.6–32.1	0.2
>14 days	6 (25)	1 (7)	3.8	0.5–28.2	0.2
Fever	10 (42)	6 (40)	1.0	0.5–2.3	0.9
Vomiting	13 (54)	1 (7)	8.1	1.2–55.9	0.003
Dehydration	11 (48)	5 (33)	1.4	0.6–3.3	0.6
<5%	5 (22)	3 (20)	1.0	0.3–3.74	1
5 to 10%	4 (17)	2 (13)	1.3	0.2–16.3	1
>10%	2 (9)	0	ND	ND	0.3
Stay duration of ≥7 days	9 (37)	7 (47)	1.2	0.7–2.0	0.6

[a] RR, relative risk.
[b] CI, 95% confidence interval.
[c] ND, not determined.

There was no association of DAEC and diarrhea, although the number of children with more than four stools per day was much higher for DAEC carriers than for controls (30% versus 7%). Published reports yield conflicting results concerning the association of DAEC with diarrhea (1–6, 9, 10). In a previous study performed by Jallat et al. (8), a significant association between the presence of DAEC in stools and diarrheal syndromes was detected. Both adults and children were included, and no age stratification was made. When only children <14 years old were considered, this association was no longer significant; diarrhea defined as ≥3 stools per 24 h for 3 days occurred in 61% of the DAEC-positive children and 47% of the DAEC-negative children (P = 0.5). Few studies of the involvement of DAEC in diarrhea including different age groups have been conducted; Gunzburg et al. showed that there was a significant association for children ≤18 months old but not for younger ones (6). Because of the limited number of patients in this study, we could not differentiate children by age and determine the statistical association between diarrhea and the presence of DAEC in each group.

Fever and dehydration were as frequent in patients as in controls. However, the two children with >10% dehydration were DAEC positive. The association of DAEC with clinical signs other than diarrhea has been poorly documented. Giron et al. (4) noted that fever and vomiting were associated with 29 and 36%, respectively, of the cases of DAEC in a study of Mayan children. It is likely that many different DAEC clones coexist, all of them exhibiting the diffusely adherent phenotype in tissue culture assays, and that only one subpopulation is pathogenic. For these reasons, the use of DNA probes, which is a more reliable method than the very subjective adhesion assay, should be a priority in DAEC detection.

Of the 24 DAEC strains, 13 hybridized with the *daaC* probe. A comparison of the general data and clinical signs for the 13 DAEC *daaC*-positive and the 11 DAEC *daaC*-negative patients revealed that only the duration of the hospital stay was significantly different. Children with DAEC *daaC*-positive strains were hospitalized for a mean duration of 15 days compared with 5 days for children with DAEC *daaC*-negative strains (P = 0.01). The carrying of DAEC was therefore significantly associated with mean illness duration, which suggests a potential pathogenic role for these bacteria. This study has been realized with a small population group (25 patients and 15 controls). Further studies including a larger and more heterogeneous population will be designed to substantiate the involvement of DAEC in clinical signs other than diarrhea associated with digestive disorders.

This work was financially supported by the Centre Hospitalier Régional Universitaire de Clermont-Ferrand, France.

We thank Valérie Livrelli and Jacques Sirot for helpful criticism and the members of Service de Pédiatrie, Hotel-Dieu, for help in collecting specimens and information.

REFERENCES

1. **Baqui, A. H., R. B. Sack, R. E. Black, K. Haider, A. Hossain, A. R. M. A. Alim, M. Yunus, H. R. Chowdhury, and A. K. Siddique.** 1992. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children <5 years of age. J. Infect. Dis. **166:**792–796.
2. **Chatkaeomorakot, A., P. Echeverria, D. N. Taylor, K. A. Bettelheim, N. R. Blacklow, O. Sethabutr, J. Seriwatana, and J. Kaper.** 1987. HeLa cell-adherent *Escherichia coli* in children with diarrhea in Thailand. J. Infect. Dis. **156:**669–672.
3. **Echeverria, P., O. Serichantalerg, S. Changchawalit, B. Baudry, M. M. Levine, F. Orskov, and I. Orskov.** 1992. Tissue culture-adherent *Escherichia coli* in infantile diarrhea. J. Infect. Dis. **165:**141–143.
4. **Giron, J. A., T. Jones, F. Millan-Velasco, E. Castro-Munoz, L. Zarate, J. Fry, G. Frankel, S. L. Moseley, B. Baudry, J. B. Kaper, G. K. Schoolnik, and L. W. Riley.** 1991. Diffuse-adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. J. Infect. Dis. **163:**507–513.
5. **Gomes, T. A. T., P. A. Blake, and L. R. Trabulsi.** 1989. Prevalence of *Escherichia coli* strains with localized, diffuse, and aggregative adherence to HeLa cells in infants with diarrhea and matched controls. J. Clin. Microbiol. **27:**266–269.
6. **Gunzburg, S. T., B. J. Chang, S. J. Elliott, V. Burke, and M. Gracey.** 1993. Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* from aboriginal children from the Kimberley region of Western Australia. J. Infect. Dis. **167:**755–758.
7. **Jallat, C., A. Darfeuille-Michaud, C. Rich, and B. Joly.** 1994. Survey of clinical isolates of diarrhoeogenic *Escherichia coli*: diffusely adhering *E. coli* strains with multiple adhesive factors. Res. Microbiol. **145:**621–632.
8. **Jallat, C., V. Livrelli, A. Darfeuille-Michaud, C. Rich, and B. Joly.** 1993. *Escherichia coli* strains involved in diarrhea in France: high prevalence and heterogeneity of diffusely adhering strains. J. Clin. Microbiol. **31:**2031–2037.
9. **Levine, M. M., V. Prado, R. Robins-Browne, H. Lior, J. B. Kaper, S. L. Moseley, R. Gicquelais, J. P. Nataro, P. Vial, and B. Tall.** 1988. Use of DNA probes and HEp-2 cell adherence assay to detect diarrheagenic *Escherichia coli*. J. Infect. Dis. **158:**224–228.
10. **Mathewson, J. J., R. A. Oberhelman, H. L. Dupont, F. J. D. L. Cabada, and E. V. Garibay.** 1987. Enteroadherent *Escherichia coli* as a cause of diarrhea among children in Mexico. J. Clin. Microbiol. **25:**1917–1919.

10a. **Robins, J., F. Greenland, and N. W. Breslon.** 1986. A general estimator for the variant of the Mantel-Haenszel odds ratio. Am. J. Epidemiol. **124:**719–723.

11. **Tacket, C. O., S. L. Moseley, B. Kay, G. Losonsky, and M. M. Levine.** 1990. Challenge studies in volunteers using *Escherichia coli* strains with diffuse adherence to HEp-2 cells. J. Infect. Dis. **162:**550–552.