

Reduced Etiological Role for Enteropathogenic *Escherichia coli* in Cases of Diarrhea in Brazilian Infants

Josias Rodrigues, Cristiane M. Thomazini, Alexandra
Morelli and Georgea C. M. de Batista
J. Clin. Microbiol. 2004, 42(1):398. DOI:
10.1128/JCM.42.1.398-400.2004.

Updated information and services can be found at:
<http://jcm.asm.org/content/42/1/398>

REFERENCES

These include:

This article cites 16 articles, 8 of which can be accessed free at:
<http://jcm.asm.org/content/42/1/398#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new
articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Reduced Etiological Role for Enteropathogenic *Escherichia coli* in Cases of Diarrhea in Brazilian Infants

Josias Rodrigues,* Cristiane M. Thomazini, Alexandra Morelli, and Georgea C. M. de Batista

Departamento de Microbiologia e Imunologia, Instituto de Biociências da UNESP em Botucatu, São Paulo, Brazil

Received 24 June 2003/Returned for modification 14 August 2003/Accepted 28 September 2003

Previously common in Brazil, enteropathogenic *Escherichia coli* (EPEC) strains of serogroups O55, O111, and O119 are now rare, while enteroadherent strains other than EPEC, belonging to serogroups such as O125, were prevalent among 126 diarrheic infants less than 1 year old who were surveyed. None of these strains had the EPEC bundle-forming pilus (*bfpA*) gene.

A number of organisms, including viral, protozoan, and bacterial agents, are associated with the etiology of infantile infectious diarrhea, a leading cause of infantile mortality and morbidity worldwide. Although representing a common species of the human intestinal microbiota, *Escherichia coli* is also considered an important bacterial agent of infantile infectious diarrhea, an association first described in the report of John Bray (2), when enteropathogenic *E. coli* (EPEC) was found as the cause of a diarrhea outbreak originating from a pediatric nursery in London, United Kingdom, in 1945. In addition to EPEC, five other diarrheagenic *E. coli* (DEC) pathotypes were later described: enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteroaggregative (EAEC), and diffusely adhering (DAEC) *E. coli*. ETEC, EAEC, and EHEC are toxin producers; EPEC, EAEC, and DAEC express characteristic adhesion patterns (localized, aggregative, and diffuse, respectively) to epithelial cells and are thus referred to as enteroadherent *E. coli*; EIEC is distinguished by the capacity to invade epithelial cells and the inability to produce toxins. Serotyping, screening for pathogenicity factors, and adherence to cultured epithelial cells comprise the main laboratorial diagnosis tools for diarrhea caused by DEC. For a detailed review of DEC, see reference 12.

In a recent survey carried out to investigate DEC prevalence in Botucatu, São Paulo State, Brazil, a region where some of the first epidemiological studies of enteropathogenic enterobacteria in Brazil were conducted (10), EAEC and rotavirus were found to be dominant, while traditional (EPEC, ETEC, EIEC, and EHEC) DEC pathotypes were absent from the stools of diarrheic children less than 13 years old (14). In order to better assess the role of EAEC in diarrhea and confirm the absence of EPEC in this population, we carried out the present study, restricting the survey to patients less than 1 year old, an age group more susceptible than others to EPEC-related diarrhea. The patient group consisted of 126 infants who attended the UNESP Hospital and the Medical Training Center of Botucatu between 1997 and 2000, with a record of at least two daily episodes of fluid stool evacuations, associated or not

with vomiting, for 3 days or more. A total of 39 age- and geographically matched controls, randomly chosen at the same time among healthy volunteers in the community, were also surveyed. The stool collection was performed with the consent of the infant's family and the approval of the Hospital Committee on Ethics in Research. According to conventional bacteriological procedures, one to three out of five colonies randomly picked on MacConkey agar stool culture from each patient was identified as *E. coli*. These isolates were stocked at -80°C in tryptic soy broth supplemented with 20% glycerol. The *E. coli* collection, encompassing 165 isolates from the 126 diarrheic infants and 66 isolates from the 39 healthy infants, was then characterized by O:H serotyping, adhesion assays, and PCR to detect the EAEC plasmid for aggregative adhesion (pAA), the EPEC bundle-forming pilus structural subunit (*bfpA*), and the *E. coli* attaching and effacing (*eae*) genes.

O and H serotyping was done by slide and tube agglutination tests, respectively, as recommended by Ewing (4). The O antisera (Probac, São Paulo, Brazil) were specific for antigens of EPEC classical O serogroups (O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158) (21). Adhesion assays were performed in HEP-2 cell monolayers for a 3-h incubation period at 37°C (14). Strains that were unable to adhere in these initial assays were submitted to 6-h incubation tests. Primers and mixture components as well as amplification conditions of PCR for *eae*, *bfpA*, and pAA are described in references 5, 8, and 16, respectively. EPEC strain E-2348/69 and EAEC strain 17-2 were used as positive controls both in the adhesion tests and in PCR.

Strains from 123 diarrheic and 39 healthy infants were tested for adhesion to HEP-2 cells, and the following phenotypes were identified: aggregative adhesion (AA), diffuse adhesion (DA), and localized-like adhesion (LLA) (Fig. 1). AA⁺ strains were found in 45 (36.6%) diarrheic and 17 (43.6%) healthy infants, and DA⁺ strains were found in 34 (27.6%) diarrheic and 14 (35.9%) healthy infants. Two LLA⁺ strains were identified, one in each of the surveyed groups. Regardless of the adhesion phenotype, strains displaying characteristic adhesion patterns were detected in higher frequencies in healthy (82%) than in diarrheic (65%) infants ($P \leq 0.05$). Still-higher figures for healthy infants' strains were observed in regard to the EPEC and EAEC genetic markers. The percentage of *eae*⁺ strains found in controls (12.8%) was over twice as high as that

* Corresponding author. Mailing address: Departamento de Microbiologia e Imunologia, IB-UNESP, Campus de Rubião Júnior, CEP 18.618-000, Botucatu, São Paulo, Brazil. Phone: 55 (14) 3811-6058. Fax: 55 (14) 3815-3744. E-mail: josias@ibb.unesp.br.

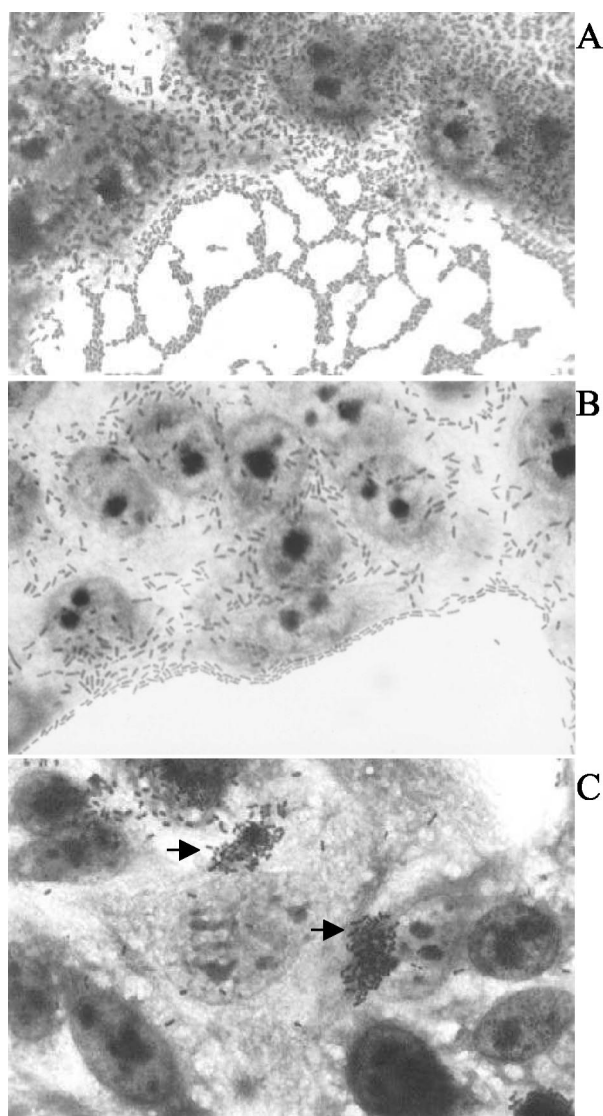


FIG. 1. Adhesion phenotypes to HEP-2 cells found among *E. coli* strains isolated from 123 diarrheic and 39 healthy infants. (A) AA; (B) DA; (C) LLA. AA⁺ and DA⁺ strains were detected after both 3 and 6 h of incubation; LLA⁺ strains were detected only after 6 h of incubation. Arrows show LLA bacterial microcolonies, usually less dense and compact than the LA clusters of typical EPEC.

of *eae*⁺ strains found in patients (5.6%). In the case of pAA, the percentage of positive strains in healthy infants was about six times that found in patients (38.5 and 6.3%, respectively). Yet, only the difference corresponding to the prevalence of pAA in healthy subjects proved to be statistically significant ($P \leq 0.001$). PCR amplification products of *eae*⁺ and pAA⁺ strains are shown in Fig. 2. Twenty-two strains were pAA⁺: 12 of them displayed AA, 5 displayed DA, and 5 were not adherent. Only five of the pAA⁺ strains were detected in patients. The gene combination *eae-bfpA* was found in only one strain, which was, however, unable to adhere to HEP-2 cells and thus not considered a typical EPEC strain; this strain was isolated from a healthy infant. Strains of the classical O serogroups were found in 26 (20.6%) of the 126 diarrheic and 6 (15.4%) of

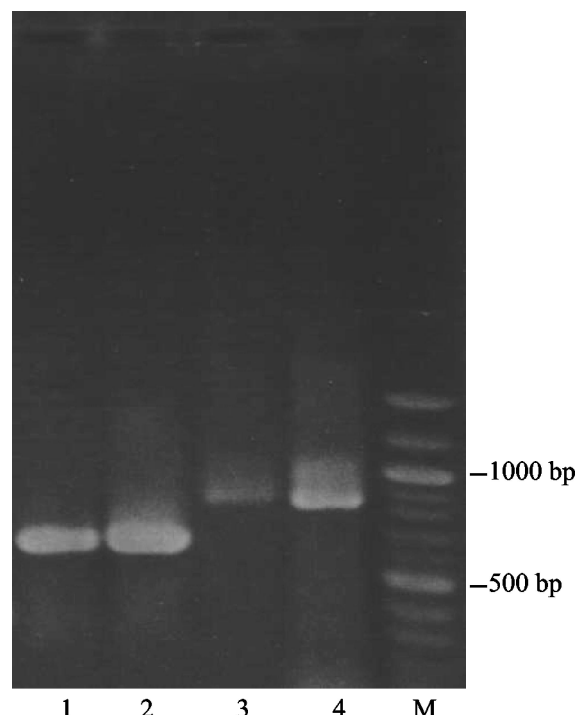


FIG. 2. PCR-amplified DNA resolved by agarose gel electrophoresis of a pAA⁺ (lane 2) and an *eae*⁺ (lane 4) *E. coli* strain. The positive-control strains 17-2 and E-2348/69 are shown in lanes 1 and 3, respectively. M, 100-bp DNA ladder.

the 39 healthy infants (Table 1). The most common serogroup was O125, with a total of seven strains detected, six of them in patients. The three adherent O125 strains showed the AA phenotype, but none of them had the pAA plasmid.

EPEC has been considered the main cause of infantile diarrhea in developing countries (1) and in some cases has been found in up to 30% of infants (6, 17). Data from the 1960s show EPEC of the O111 serogroup as representing 70% of the *E. coli* isolates from diarrheic children living in Botucatu (11). In 1996, the EPEC concept was refined in order to accommodate molecular and genetic pathogenesis features uncovered in previous years (9). The *bfpA* and *eae* genes associated with the capacity to display localized adhesion (LA) and to induce the attaching and effacing lesions in epithelial cells and with failure to produce Shiga cytotoxins are the differential markers of EPEC strains (9). Since not all of the classical O serogroups have strains with these features, the number of recognized EPEC serogroups was reduced. It was also observed that some of the classical O serogroups might include more than one DEC pathotype, which occasionally can be identified by its O:H type (3, 7, 15). For example, serotypes O55:H6, O86:H34, O111:H2, and O119:H6 usually mark EPEC strains (19), whereas O111:H10 and O111:H12 are EAEC (3). In this survey, strains of serogroups O55, O111, O114, O119, and O142 as well as six out of the seven O125 strains were isolated only from diarrheic infants (Table 1). Yet, none of them showed the typical EPEC markers, i.e., LA, *eae*, and *bfpA*. Most of these strains showed an AA usually not correlated with the presence of pAA (Table 1).

In the past, the most prevalent EPEC serogroups in Brazil

TABLE 1. Adhesion phenotypes and genetic pathogenicity markers of *E. coli* strains in relation to O:H serotypes found among 126 diarrheic and 39 nondiarrheic infants

Serotype ^a	Strains from infants ^b :	
	Diarrheic	Nondiarrheic
O26:H ⁻	NA [<i>eae</i>]	
O55:H ⁻ (2)	NA, AA	
O55:H? (2)	NA, AA	
O86:H? (3)	DA, AA	AA
O111:H?	NA	
O114:H ⁻	NA	
O114:H?	NA [pAA]	
O114:H10	DA	
O114:H4	AA	
O119:H2	AA [pAA]	
O125:H ⁻ (3)	AA (2), NT	
O125:H? (2)	AA	NA
O125:H12	NT	
O125:H21	NA [<i>eae</i> -pAA]	
O126:H ⁻	DA	
O126:H?		AA [pAA]
O127:H?		DA
O128:H?		AA
O142:H ⁻	DA	
O142:H?	NA	
O142:H10 (2)	AA, NA	
O158:H? (3)	AA, NA	AA
Total no./%	26/20.6	6/15.4

^a H⁻ and H? indicate serotypes of nonmotile strains and serotypes with flagellar antigens other than H2, H4, H6, H7, H8, H9, H10, H12, and H21, respectively. Numbers in parentheses are numbers of strains for serotypes with multiple strains.

^b Genetic pathogenicity markers are in brackets: *eae*, *E. coli* attaching and effacing gene; pAA, plasmid for AA. NA, nonadherent; NT, not tested.

were O55, O111, and O119 (20). O111 strains were dominant particularly among the population that we have studied (11). Though in those times *E. coli* isolates were not screened for the presence of EPEC pathogenicity markers, comparative (13) and epidemiological (18) analyses demonstrate that these serogroups include a high proportion of EPEC strains. In this survey, we detected a low number of strains of serogroups O55, O111, and O119, which either were nonmotile or did not react with antisera specific for the flagellar antigens of common EPEC serotypes, such as O55:H6, O111:H2, and O119:H6 (Table 1). Thus, by employing currently recognized diagnosis assays, our results reveal an evident decline in the incidence of EPEC strains and the prevalence of a heterogeneous group of AA⁺ and DA⁺ enteroadherent *E. coli* strains in Botucatu. Since they were found at a higher frequency in healthy infants than in patients, it is likely that at least some of them may belong to normal microbiota clones sharing the adhesion ability and the pAA marker of pathogenic DAEC and EAEC. On the other hand, the specific association of some serogroups with diarrheic patients is suggestive of an enteropathogenic role for some enteroadherent strains of particular serogroups, such as O125. In order to better evaluate the extent of the involvement of these strains with diarrhea and to know whether they are emerging DEC pathotypes that have replaced

previously common EPEC serogroups, new studies aiming to characterize putative new virulence factors and to identify enteroadherent *E. coli* clones are necessary.

This work was supported by FAPESP grant no. 1997/07733-9 awarded to J.R.

REFERENCES

- Albert, M. J. 1996. Epidemiology of enteropathogenic *Escherichia coli* infection in Bangladesh. *Rev. Microbiol.* **27**(Suppl. 1):17-20.
- Bray, J. 1945. Isolation of antigenically homogeneous strains of *Bact. coli neopolitana* from summer diarrhoea of infants. *J. Pathol. Bacteriol.* **57**: 239-247.
- Campos, L. C., T. S. Whittam, T. A. T. Gomes, J. R. C. Andrade, and L. R. Trabulsi. 1994. *Escherichia coli* serogroup O111 includes several clones of diarrheagenic strains with different virulence properties. *Infect. Immun.* **62**: 3282-3288.
- Ewing, W. H. 1986. The genus *Escherichia*, p. 93-134. In W. H. Ewing (ed.), *Identification of Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
- Gannon, V. P. J., M. Rashed, R. K. King, and E. J. Thomas. 1993. Detection and characterization of the *eae* gene of Shiga-like toxin-producing *Escherichia coli* using the polymerase chain reaction. *J. Clin. Microbiol.* **31**:1268-1274.
- Gomes, T. A. T., M. A. M. Vieira, I. K. Wachsmuth, P. A. Blake, and L. R. Trabulsi. 1989. Serotype-specific prevalence of *Escherichia coli* strains with EPEC adherence factor genes in infants with and without diarrhea in São Paulo, Brazil. *J. Infect. Dis.* **160**:131-135.
- Gonçalves, A. G., L. C. Campos, T. A. T. Gomes, J. Rodrigues, V. Sperandio, T. S. Whittam, and L. R. Trabulsi. 1997. Virulence properties and clonal structures of strains of *Escherichia coli* O119 serotypes. *Infect. Immun.* **65**: 2034-2040.
- Gunzburg, S. T., N. G. Tornieporth, and L. W. Riley. 1995. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *J. Clin. Microbiol.* **33**:1375-1377.
- Kaper, J. B. 1996. Defining EPEC. *Rev. Microbiol.* **27**(Suppl. 1):130-133.
- Montelli, A. C., and L. R. Trabulsi. 1970. Diarréias causadas por *Shigella*, *Salmonella* e *Escherichia coli* enteropatógenas no município de Botucatu, São Paulo. *Rev. Assoc. Med. Bras.* **16**:23-26.
- Montelli, A. C. 1972. Diarréias por enterobactérias. Estudo bacteriológico, clínico e epidemiológico. Ph.D. thesis. Faculdade de Medicina da UNESP de Botucatu, São Paulo, Brazil.
- Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11**:142-201.
- Robins-Browne, R. M., W. C. Yam, L. E. O'Gorman, and K. A. Bettelheim. 1993. Examination of archetypal strains of enteropathogenic *Escherichia coli* for properties associated with bacterial virulence. *J. Med. Microbiol.* **38**:222-226.
- Rodrigues, J., V. C. Acosta, J. M. G. Candeias, L. O. Souza, and F. J. C. Filho. 2002. Prevalence of diarrheagenic *Escherichia coli* and rotavirus among children from Botucatu, São Paulo state, Brazil. *Braz. J. Med. Biol. Res.* **35**:1311-1318.
- Rodrigues, J., I. C. A. Scaletsky, L. C. Campos, T. A. T. Gomes, T. S. Whittam, and L. R. Trabulsi. 1996. Clonal structure and virulence factors in strains of *Escherichia coli* of the classic serogroup O55. *Infect. Immun.* **64**:2680-2686.
- Schmidt, H., C. Knop, S. Franke, S. Aleksic, J. Heesemann, and H. Karch. 1995. Development of PCR for screening of enteroaggregative *Escherichia coli*. *J. Clin. Microbiol.* **33**:701-705.
- Trabulsi, L. R. 1985. Epidemiology of diarrhoeal diseases in South America, p. 121-125. In S. Tzipori (ed.), *Infectious diarrhoea in the young*. Elsevier Biomedical Press, Amsterdam, The Netherlands.
- Trabulsi, L. R., L. C. Campos, T. S. Whittam, T. A. T. Gomes, J. Rodrigues, and A. G. Gonçalves. 1996. Traditional and non-traditional enteropathogenic *Escherichia coli* serogroups. *Rev. Microbiol.* **27**(Suppl. 1):1-6.
- Trabulsi, L. R., R. Keller, and T. A. T. Gomes. 2002. Typical and atypical enteropathogenic *Escherichia coli*. *Emerg. Infect. Dis.* **8**:508-513.
- Trabulsi, L. R., M. R. F. Toledo, J. Murahovsky, U. Fagundes-Neto, and J. A. N. Cadeias. 1985. Epidemiology of infantile bacterial diarrheal disease in Brazil, p. 25-36. In Y. Takeda and T. Miwatani (ed.), *Bacterial diarrheal diseases*. K. T. K. Scientific Publishers, Tokyo, Japan.
- World Health Organization. 1987. Programme for control of diarrhoeal diseases. Manual for laboratory investigations of acute enteric infections. World Health Organization, Geneva, Switzerland.