Genotypic and phenotypic analysis of diarrheagenic Escherichia coli strains isolated from Brazilian children living in low socioeconomic level communities

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Abstract

Background: Childhood diarrheal diseases remain highly endemic in developing areas of Brazil. The importance of Escherichia coli among children with diarrhea in these areas was unknown. This study determined the prevalence of different E. coli categories in symptomatic and asymptomatic children from low socioeconomic level rural communities in southeastern Brazil.

Methods: A total of 560 stool samples were collected from 141 children with diarrhea (< 10 years) and 419 apparently healthy controls who resided in 23 communities. E. coli isolates (n = 1943) were subjected to two multiplex PCRs developed for the detection of enteropathogenic E. coli (EPEC), enteroaggregative E. coli (EAEC), diffusely adherent E. coli (DAEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), and Shiga toxin-producing E. coli (STEC). Strains were also examined for the presence of EPEC, EAEC, and DAEC by assays of adhesion to HEp-2 cells and by hybridization with specific DNA probes.

Results: Diarrheagenic E. coli strains were isolated from 253 (45.2%) children, and were associated with diarrhea in children aged < 5 years (p < 0.001). EAEC (20.9%), DAEC (11.6%), EPEC (9.3%) were the most frequent pathotypes, followed by ETEC (2.7%), EIEC (0.5%), and STEC (0.2%). Depending of the assay, EPEC, EAEC, and DAEC (collectively termed enteroadherent E. coli) strains were isolated in 45% to 56% of diarrhea cases, a significantly higher incidence than in controls (P < 0.05). Individually, only DAEC showed significant association with diarrhea (p < 0.05), particularly in children aged 2–5 years.

Conclusion: This study indicates that enteroadherent E. coli is an important cause of diarrhea in children living in low socioeconomic level communities in southeastern Brazil. Our results reveal that the PCR1 assay is an excellent tool for the identification of EAEC and DAEC.

Background

Diarrheal disease remains a major public health problem in developing countries, and is responsible for high morbidity and mortality among children under 5 years [1,2]. In Latin America, diarrheal diseases are responsible for ~10% of childhood deaths [3]. Escherichia coli strains are among the most important bacterial causes of childhood diarrhea.

Diarrheagenic E. coli (DEC) strains can be divided into six main categories on the basis of distinct epidemiological and clinical features, and specific virulence determinants [4]: enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enterohemorrhagic E. coli (EHEC) or Shiga-toxin producing E. coli (STEC), enteroaggregative E. coli (EAEC), and diffusely adherent E. coli (DAEC). EPEC is classified into typical and atypical strains based on the presence of the EPEC adherence factor (EAF) plasmid [4]. Although DEC strains are of public health relevance, they are not routinely sought as enteric...
localed 256 km north of the city of Espirito Santo, Brazil.

Brazil using phenotypic and genotypic diagnostic tests.

mine the importance of EPEC, EAEC and DAEC in rural

a prospective active diarrheasurveillance study to deter-

EAEC and DAEC in children with diarrhea. We conducted

plex PCR assay for the detection of EPEC, EAEC, DAEC,

order to simply diagnosis, we set up a two-reaction multi-

results with a high sensitivity and a high specificity. In

characteristics, and molecular methods [4]. Among these,

PCR is a commonly used method that gives rapid, reliable

h to the laboratory at the Universidade Federal do Espírito

in the Lab. Specimen collection and processing

Centers with low socioeconomic status

quent active diarrhea surveillance study to deter-

of EPEC, EAEC and DAEC in rural communities with low socioeconomic level in southeastern Brazil using phenotypic and genotypic diagnostic tests.

Methods

Ethical clearance, study site and population

The study was approved by the Ethical Committee of Re-

are dominantly agricultural, and the population

lives in slum conditions, with no access to potable water

set of primers except for the

The “quilombola” communities are composed of descen-

of slaves who escaped from slave plantations that

that existed in Brazil before abolition in 1880. These communiti-

are predominately agricultural, and the population

with no access to potable water or sewage facilities. The households were visited once a

week by nurses and trained community health workers. At each visit, the mother was asked a standard series of ques-

ions on her child’s stool frequency, consistency, and

physical nature. If a child had diarrhea, his or her hydration

status was assessed. Generally fluid and nutritional

management was recommended. Diarrhea was defined as

the occurrence of three or more loose, liquid, or watery

set of primers except for the

ipaH primers, which used

10 μM. The reactions were run in a thermal cycler (model system 2400; Perkin-Elmer Corporation, Nor-

walk, Conn.) with the following cycling conditions: 94°C

for 5 min, 40 cycles of denaturation at 95°C for 1 min,

annealing at 58°C (assay 1) or 50°C (assay 2) for 1 min

and primer extension at 72°C for 2 min followed by a

final extension at 72°C for 7 min. PCR products (10 μL)

were visualized after electrophoresis in 2% agarose gels

in Tris-borate-EDTA buffer and ethidium bromide stain-

ing. In all assays, a mixture of DNA from the prototype

EPEC E2348/69, EAEC 042, DAEC C1845, ETEC H10407,

EIEC EDL1284, and STEC EDL931 strains served as the

positive control, while E. coli K-12 DH5α was the negative

control [4].

Multiplex PCR assays

All E. coli isolates were subjected to two multiplex PCRs,

as previously described, with some modifications [5].

PCR1 assay contained a primer mix for the detection of

the following virulence markers: E. coli attaching and ef-

facing (eae) gene (for detection of typical and atypical

EPEC), EAF plasmid (for detection of typical EPEC

strains), and the antiaggregation protein transporter

gene (aat; previously referred to as CVD432 or the AA

probe) (for detection of EAEC strains). Primers specific

for afa/daa for the detection of DAEC strains were sub-

sequently included into this multiplex PCR. PCR2 assay

contained primers specific for elt and est (enterotoxins of

ETEC), ipaH (invasion plasmid antigen H found in

EIEC and Shigella), and stx1 and stx2 (Shiga toxins 1, 2

and variants of STEC). PCR1 assay identified EAEC,

DAEC, and tEPEC by the presence of eae and bfpA, and

aEPEC by the presence of only eae. PCR2 assay identi-

fied ETEC, EIEC, and STEC.

Three to six bacterial colonies from each stool sample

were pooled for template DNA preparation immediately

prior to PCR testing, suspended in 300 μL of sterile

water, and boiled for 10 min. A 5-μL aliquot of this sus-

pension was added to 50 μL of the PCR mixture

(50 mM KCl, 10 mM Tris–HCl [pH 8.3], 1.5 mM MgCl2,

2 mM of each deoxynucleoside triphosphate), 1.5 U

of AccuPrime Taq DNA polymerase, and 5 μM of each

set of primers except for the ipaH primers, which used

10 μM. The reactions were run in a thermal cycler

(model system 2400; Perkin-Elmer Corporation, Nor-

walk, Conn.) with the following cycling conditions: 94°C

for 5 min, 40 cycles of denaturation at 95°C for 1 min,

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positive control, while E. coli K-12 DH5α was the negative

control [4].

Specimen collection and processing

Stool samples were collected and placed in Cary-Blair

transport medium, and transported in iced boxes within 4

h to the laboratory at the Universidade Federal do Espirito

Santo. Samples were inoculated onto the surface of

MacConkey and Hektoen agars for the selection of

E. coli, Shigella, and Salmonella isolates. After incuba-

tion for 24 h at 37°C, four lactose-fermenting colonies

with typical E. coli morphology, and two non-lactose-

fermenting colonies were subjected to biochemical

tests for identification. In addition, samples were ana-

lyzed for the presence of Giardia lamblia, Entamoeba

hystolytica, and Cryptosporidium, using standard methods

[10]. All E. coli strains were maintained in nutrient

glans at room temperature.
HEp-2 adherence assay

*E. coli* isolates were subjected to HEp-2 adherence tests by the method originally described by Scaletsky et al. [11], with slight modifications. Briefly, monolayers of 10^5 HEp-2 cells were grown in Dulbecco modified Eagle medium containing 10% fetal bovine in 24-well tissue culture plates (Falcon Becton Dickinson). Bacterial strains were grown statically in 2 ml of brain heart infusion for 16–18 h. The monolayers were infected with ~3 X 10^7 bacteria (20 μl of bacterial cultures added to 1 ml of DMEM) and incubated at 37°C for 3 h. The infected monolayers were washed with sterile PBS, fixed with methanol, stained with Giemsa stain, and examined for localized adherence (LA), diffuse adherence (DA), and aggregative adherence (AA).

DNA hybridization

*E. coli* isolates were tested by colony hybridization with the following DNA probes: EPEC adherence factor EAF (1-kb *BamHI-Sall* fragment of pMAR2 [12], E. coli attaching and effacing gene encoding intimin (*eae*) (1-kb *KpnI-Sall* fragment of pCVD434 [13], CVD432 (the nucleotide sequence of the *EcoRI-PstI* fragment of pCVD432 of EAEC) [14], and daac (associated with the biogenesis of DAEC F1845 adhesin) (390 bp *PstI* fragment of pSLM852 [15]). DNA probes were prepared from recombinant plasmids containing the DNA probe fragments as inserts. Plasmids were extracted by the method of Birnboim and Doly [16] and digested with appropriate restriction endonucleases, and the appropriate restriction fragments were purified by gel extraction. The DNA fragments were labeled by a random primer extension kit (Rediprime DNA labeling system; Amersham) with [α-32P]dCTP. Colony blots were prepared with Whatman 541 filter papers which were then processed and hybridized under string conditions as described previously [17].

Statistical analysis

The statistical analyses were performed using the SPSS version 17.0 (SPSS Inc., Chicago, IL). A sample size of 100 cases and 300 controls was estimated to have 80% power to detect the symptoms associated with infection, assuming a prevalence of 15% in controls and a 5% significance level. Statistical differences were evaluated by chi-square or Fisher's exact tests. A p value < 0.05 was considered statistically significant.

Results

Between August 2007 and September 2008, 560 stool samples were collected from 141 children with diarrhea and 419 healthy controls who resided in 23 communities. Among the population studied, 150 (26.8%) children were aged less than 2 years; 216 (38.6%) and 194 (34.6%) were aged 2–5 years and 6–10 years, respectively. The overall sex distribution was 296 (52.8%) male and 264 (47.1%) female.

*E. coli* isolates (n = 1,943) were categorized into different pathotypes based on the results of two multiplex PCRs (Table 1). Diarrheagenic *E. coli* strains were isolated from 253 (45.2%) children, and were associated with diarrhea in children aged < 5 years (p < 0.001). EAEC (20.9%), DAEC (11.6%), aEPEC (8.7%) were the most frequent pathotypes, followed by LT-ETEC (2.3%), tEPEC (0.5%), EIEC (0.5%), LT/ST ETEC (0.4%), and STEC (0.2%). Other enteric pathogens isolated were *Shigella* (0.7%), *Salmonella* (0.2%), *Giardia lamblia* (4.1%), and *Entamoeba histolytica* (0.9%). Mixed infections were presented in 22 (15.6%) cases and 12 (2.9%) controls (p < 0.05).

*E. coli* isolates were further characterized into EPEC, EAEC, and DAEC pathotypes by HEP-2 adherence pattern and DNA probes. The results were compared with the PCRI assay (Table 2). Of 145 isolates which yielded the AA pattern, 117 were detected by PCRI, and 99 of these strains reacted with the CVD432 probe; 18 EAEC isolates gave a positive-PCR but probe negative result. Similarly, PCRI detected 65 of 104 isolates that yielded the DA pattern, and 57 of these strains reacted with the daac probe; only 8 isolates gave a positive-PCR but probe negative result.

The combined incidence of EPEC, EAEC, and DAEC (collectively termed enteroadherent *E. coli*) isolates from diarrhea cases and controls is shown in Table 3. Enteroadherent *E. coli* strains were significantly associated with diarrhea, whether detected by phenotypic HEP-2 cell assay or by genotypic assays (p < 0.05). Depending on the assay used, enteroadherent *E. coli* strains were isolated in 45% to 56% of cases, a significantly higher incidence that in controls (p < 0.05). Individually, only DAEC strains, detected either by adherence pattern or by PCRI or by DNA probe, were significantly associated with diarrhea (p < 0.05), particularly in children aged 2–5 years.

Discussion

Diarrheal disease remains an important public health problem for children in developing areas of Brazil. The importance of *E. coli* as a cause of diarrhea and its attributable fraction to the diarrhea prevalence in these areas was unknown. The present study was performed to determine the prevalence of different diarrheagenic *Escherichia coli* categories in symptomatic and asymptomatic children living in low socioeconomic rural communities in southeastern Brazil.

We employed a two-reaction multiplex PCR assay for the detection of the six *E. coli* pathotypes. The PCR 1 contained primers for amplification of *eae*, *EAF*, *aat*, and *afa/daa* genes for identification of EPEC, EAEC, and
DAEC. The PCR 2 contained primers for amplification of LT, ST, Inv, and Stx genes for detection of ETEC, EIEC, and STEC. We were able to validate the PCR1 with HEp-2 cell adherence assays and specific DNA probes. The different combination of adherence patterns and PCR primers showed that the HEp-2 adherence assay remains the "gold standard" for detection of EAEC and DAEC. Compared with DNA hybridization, our results showed that PCR1 assay could be used instead of the DNA probes as a screening method for "typical" EAEC (aat+) and "typical" DAEC (afa/daa+) strains in the clinical laboratory [17].

Our results show a high proportion of EPEC, EAEC, and DAEC (collectively termed enteroadherent E. coli) accounting for 92.4% of DEC. EAEC was the most prevalent pathotype in both diarrhea cases and controls, in contrast with several studies showing an association with diarrhea [18,19]. In recent studies conducted in different urban centers of Brazil, EAEC strains were found to be dominant and associated with diarrhea [6-9]. By using the CVD432 probe, we found a sensitivity and specificity similar to those of the CVD432 probe.

| Pathotype | Diagnostic test | Cases | Controls | p value |
|-----------|----------------|-------|----------|---------|
| EPEC      | PCR            | 79 (56) | 155 (36.9) | 0.0001 |
|           | DNA probe      | 64 (45.4) | 142 (33.9) | 0.0156 |
|           | HEp-2 adhesion | 78 (55.3) | 174 (41.5) | 0.0046 |
| EAEC      | PCR            | 36 (25.5) | 81 (19.3) | 0.1210 |
|           | DNA probe      | 27 (19.1) | 72 (17.2) | 0.6107 |
|           | HEp-2 adhesion | 40 (28.4) | 105 (25) | 0.4385 |
| DAEC      | PCR            | 26 (18.4) | 39 (9.3) | 0.0057 |
|           | DNA probe      | 21 (14.9) | 36 (8.6) | 0.0369 |
|           | HEp-2 adhesion | 35 (24.8) | 69 (16.5) | 0.0336 |
| EPEC      | PCR (eae)      | 16 (11.3) | 33 (8.3) | 0.2280 |
|           | PCR (bfpA)     | 1 (0.7) | 2 (0.5) | 1.0000 |
|           | eae probe      | 15 (10.6) | 32 (7.6) | 0.2923 |
|           | EAF probe      | 1 (0.7) | 2 (0.5) | 1.0000 |
|           | HEp-2 adhesion | 1 (0.7) | 2 (0.5) | 1.0000 |

Table 2 Detection of enteroadherent E. coli (ECC) isolated from cases and controls by genotypic and phenotypic diagnostic tests

| Diagnostic test | EEC (n=560) | EPEC | EAEC | DAEC |
|-----------------|-------------|------|------|------|
| PCR assay       | 234 (41.8)  | 52 (9.3) | 117 (20.9) | 65 (11.6) |
| DNA probe       | 206 (36.8)  | 50 (8.9) | 99 (17.7) | 57 (10.2) |
| HEp-2 adhesion  | 252 (45)    | 3 (0.5) | 145 (25.9) | 104 (18.6) |

Table 1 Isolation of pathogens (DEC strains by multiplex PCR) from the stools of children with diarrhea (cases) and children without diarrhea (controls)

| Pathogen         | Children aged <2 years | Children aged 2–5 years | Children aged 6–10 years | Total no. of children |
|------------------|-------------------------|--------------------------|--------------------------|----------------------|
|                  | Cases (n = 40)          | Controls (n = 110)       | Cases (n = 53)           | Controls (n = 163)   |
| aEPEC            | 7 (17.5)                | 13 (11.9)                | 5 (9.4)                  | 13 (7.9)             |
| tEPEC            | 1 (2.5)                 | 0                        | 0                        | 2 (4.2)              |
| EAEC             | 20 (50)                 | 25 (22.7)                | 12 (22.6)                | 33 (20.2)            |
| DAEC             | 5 (12.5)                | 10 (9.1)                 | 15 (28.3)                | 18 (11.0)            |
| LT-ETEC          | 2 (5.0)                 | 1 (0.9)                  | 3 (5.7)                  | 2 (1.2)              |
| LT/ST-ETEC       | 0                      | 0                        | 1 (1.9)                  | 0                    |
| EIEC             | 0                      | 0                        | 2 (3.8)                  | 0                    |
| STEC             | 0                      | 0                        | 0                        | 1 (0.7)              |
| Shigella spp.    | 35 (87.5)              | 49 (44.5)                | 38 (71.7)                | 67 (41.1)            |
| Salmonella spp.  | 0                      | 1 (0.9)                  | 2 (1.4)                  | 1 (0.6)              |
| Giardia lamblia  | 6 (15)                 | 5 (4.5)                  | 3 (5.7)                  | 8 (4.9)              |
| Entamoeba histolytica | 0 | 0 | 1 (1.9) | 3 (1.8) | 0 | 5 (0.9) |
associated with diarrhea, especially in children aged 2–5 years. Similarly, in a prospective cohort study in a low socioeconomic level peri-urban community in Santiago, Chile [20], DAEC was found in a large proportion of diarrhea cases. In Mayan children in Mexico [21] and aboriginal children in Australia [22], DAEC strains were also predominant and associated with diarrhea. Taken together, these findings suggest that DAEC may be an important enteric pathogen in children living in endemic areas. Our results also support the evidence from studies showing an association of DAEC with age-dependent diarrhea [20,22].

In this study, aEPEC was more common than tEPEC, with concordance with current data suggesting that aEPEC is more prevalent than tEPEC in both developed and developing countries [4,23]. In Brazil, aEPEC has been increasingly reported and was recently implicated as a cause of diarrhea in different urban centers of Brazil [6–9]. Although differences in the population studied may exist, the prevalence of aEPEC in this and other studies underscores the emergence of aEPEC strains in Brazil.

The prevalence of ETEC was low, in agreement with other studies performed in different parts of Brazil [6–9]. EIEC and STEC were infrequently isolated, suggesting a less important role in diarrhea in Brazilian children [6–9].

Conclusion
We conclude that enteroadherent E. coli strains are involved in a significant proportion of diarrhea cases among children from low socioeconomic level communities. Apart from DAEC whose pathogenic role in diarrhea is still controversial, EAEC was the predominant E. coli pathotype. This should be taken as an alert for public health policies since the EAEC pathotype may harbor other very threatening virulence factors such as Shiga toxins. In addition, this study demonstrates that PCR1 assay is an excellent tool for the identification of EAEC and DAEC.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
LCS and SSK designed the study. FV were responsible for collection of specimens and clinical information. Laboratory investigations and data analysis were performed by DML, TBS, and MMV. ICSA assisted in the development of the research proposal and preparation of manuscript. All authors read and approved the final manuscript.

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References
1. Guerant RL, Hughes JM, Lima NL, Crane J: Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Rev Infect Dis 1990, 12(Suppl 1):S41–S50.
2. Parashar U, Umesh D, Bresee JS, Joseph S, Glass RI, Roger T: The global problem burden of diarrhoeal disease in children. Bull World Health Organ 2003, 81(4):236.
3. Organizacão Panamericana da Saúde/Organization Mondial de la Salud. Boletin AIEP 1997, 27:135–144.
4. Nataro JP, Kaper JB: Diarrheagenic Escherichia coli. Clin Microbiol Rev 1998, 11(1):142–201.
5. Aranda IRS, Fagundes-Neto U, Scaletsky ICA: Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic Escherichia coli and Shigella spp. J Clin Microbiol 2004, 42:5849–5853.
6. Araujo JM, Tabarelli GF, Aranda KS, Fabbricotti SH, Mendes CMF, Fagundes-Neto U, Scaletsky ICA: Typical enteroaggregative and atypical enteropathogenic types of Escherichia coli are the most prevalent diarrhea-associated pathotypes among Brazilian children. J Clin Microbiol 2007, 45:3396–3399.
7. Franzolin MR, Alves RC, Keller R, Gomes TAT, Beutin L, Baneto ML, Mikoy C, Strina A, Ribeiro H, Trabulsi LR: Prevalence of diarrheagenic Escherichia coli in children with diarrhea in Salvador, Bahia, Brazil. Mem Inst Oswaldo Cruz 2007, 102:359–363.
8. Sapiro LC, Sadovsky ADI, Segui PN, Saikw KD, Kitagawa SMS, Pereira FEL, Fagundes-Neto U, Scaletsky ICA: Age-specific prevalence of diffusely adherent Escherichia coli in Brazilian children with acute diarrhea. J Med Microbiol 2008, 57:359–363.
9. Moreno AC, Fernandes Filho A, Gomes TAT, Ramos STS, Montemor LPG, Tavares VC, Santos Filho L, Irino K, Martinez MB: Etiology of childhood diarrhea in the northeast of Brazil: significant emergent diarrheal pathogens. Diag Microbiol Infect Dis 2010, 66:50–57.
10. Murray PR, Baron MA, Pfaller MA, Tenover FC, Yolken RH: Manual of Clinical Microbiology. Washington: ASM Press; 1999.
11. Scaletsky ICA, Silva MLM, Trabulsi LR: Distinctive patterns of adherence of enteropathogenic Escherichia coli to HeLa cells. Infect Immun 1984, 45:534–536.
12. Nataro JP, Baldini MM, Kaper JB, Black RE, Bravo N, Levine MW: Detection of an adherent factor of enteropathogenic Escherichia coli with a DNA probe. Pediatr Infect Dis J 1989, 8:360–365.
13. Jerse AE, Jun Y, Tall BD, Kaper JB: A genetic locus of enteropathogenic Escherichia coli necessary for the production of attaching and effacing lesions on tissue culture cells. Proc Natl Acad Sci USA 1990, 87:7839–7843.
14. Baudry B, Savariraj SJ, Vial P, Kaper JB, Levine MW: A sensitive and specific DNA probe to identify enterogaggregative E. coli, a recently discovered diarrheal pathogen. J Infect Dis 1990, 161:1249–1251.
15. Birge SS, Clausen CR, Liu W, Moses SJ: Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhoea-associated Escherichia coli to HEp-2 cells. J Bacteriol 1989, 171:4281–4289.
16. Bimboic HM, Doly J: A rapid extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 1970, 7:1513–1523.
17. Scaletsky ICA, Fabbricotti SH, Aranda KR, Morais MM, Fagundes-Neto U: Comparison of DNA hybridization and PCR assays for detection of putative pathogenic enterohaemorrhagic Escherichia coli. J Clin Microbiol 2002, 40:1254–1258.
18. Nguyen TV, Le Van P, Huyn L, Gia KN, Weintraub A: Detection and characterization of diarrheagenic Escherichia coli from young children in Hanoi, Vietnam. J Clin Microbiol 2005, 43:755–760.
19. Sarantuya J, Nishi J, Wakimoto N, Erdene N, Nataro JP, Sheikh J, Inshwata M, Manago K, Tokuda K, Yoshinaha M, et al: Typical enterohaemorrhagic Escherichia coli is the most prevalent pathotype among E. coli strains causing diarrhea in Mongolian children. J Clin Microbiol 2004, 42:133–139.
20. Levine ML, Ferreccio C, Prado V, Cayazzo PA, Martinez J, Maggi L, Baldini MM, Martin W, Maneval D, Kay B, Guers LM, Lior H, Wasserman SS, Nataro JP: Epidemiological studies of Escherichia coli diarrheal infections in a
low socioeconomic level peri-urban community in Santiago, Chile.

Am J Epidemiol 1993, 138(10):849–869.

21. Girón JA, Jones T, Millán-Velasco F, Castro-munoz E, Zarate L, Fry J, Frankel G, Mosely SL, Baudry B, Kaper JB, Schoolnik GK, Riley LW: Diffuse-adhering Escherichia coli (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. J Infect Dis 1991, 163:507–513.

22. Gunzburg ST, Chang SJ, Elliot SJ, Burke V, Gracey M: Diffuse and enteroaggregative patterns of adherence of enteric Escherichia coli from aboriginal children from the Kimberley region of Western Australia. J Infect Dis 1993, 167:755–758.

23. Ochoa TJ, Ecker L, Baletta F, Mispireta ML, Gil AI, Conteras C, Molina M, Amemiya I, Verastegui H, Hall EB, Cleary TG, Lanata CF: Age-related susceptibility to infection with diarrheagenic Escherichia coli among infants from periurban areas in Lima, Peru. Clin Infect Dis 2009, 49:1694–1702.

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