Epidemiol. Infect. (2002), **129**, 49–55. © 2002 Cambridge University Press
DOI: 10.1017/S0950268802007136 Printed in the United Kingdom

Combinations of putative virulence markers in typical and variant enteroaggregative *Escherichia coli* strains from children with and without diarrhoea

W. P. ELIAS[1], A. P. UBER[1], S. K. TOMITA[2], L. R. TRABULSI[1] AND T. A. T. GOMES[2]*

[1] *Laboratório Especial de Microbiologia, Instituto Butantan, São Paulo, Brazil*
[2] *Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Rua Botucatu 862, São Paulo, SP, 04023-062, Brazil*

(*Accepted 1 March 2002*)

SUMMARY

Enteroaggregative *Escherichia coli* (EAEC) is defined by the ability to produce aggregative adherence (AA) to cultured cells. We analysed 128 EAEC strains, isolated from children with and without diarrhoea, regarding the presence of 11 EAEC virulence genes. Seventy strains carried and 58 lacked the EAEC probe sequence; 17 probe positive and 31 probe negative strains showed variations in the AA pattern. All EAEC probe positive strains carried at least one EAEC marker; *aspU* (94·3%), *irp2* (91·4%), and *aggR* (74·3%) were the most prevalent. Conversely, among the EAEC probe negative strains, 41·4% were devoid of any marker and *astA* predominated (44·8%). No significant statistical difference in the prevalence of any marker between cases and controls in both EAEC probe groups or AA variants was found. We suggest that the EAEC probe positive strains may have a higher pathogenic potential or alternatively, EAEC probe negative strains may harbour virulence factors as yet undescribed.

INTRODUCTION

Enteroaggregative *Escherichia coli* (EAEC) is an important agent of persistent diarrhoea in the developing world and of outbreaks of diarrhoea in the developed world [1]. EAEC strains are identified by their ability to produce an aggregative adherence (AA) pattern to HEp-2 and HeLa cells in culture, which consists of bacterial attachment to the cells and the intervening cell growth surface in a stacked-brick lattice [2]. Variations of the AA phenotype have been described, which include bacteria showing AA predominantly to the coverslip (AAcs) or predominantly to the epithelial cells (AAcel) [3–5]. Moreover, some EAEC strains promote cell-detachment (CD) during the adherence assay, a phenomenon that has been associated with the production of α-haemolysin [6].

EAEC strains generally harbour a high molecular weight plasmid (pAA) associated with AA [1], from which a DNA fragment has been obtained and employed as a diagnostic probe for the category [7]. However, EAEC strains lacking the EAEC probe sequence have been reported [1, 3, 4]. As a consequence, adherence to HeLa or HEp-2 cells remains the gold standard assay to identify EAEC [1].

Although several putative virulence factors have been identified in EAEC prototype strains, their role in pathogenesis has not been elucidated [1, 8]. Some of these EAEC putative virulence genes (EAEC markers) have been located on pAA and others on the chromosome of prototype EAEC strains. pAA located markers include those encoding proteins involved in the biogenesis of the aggregative adherence fimbria I (AAF/I) and II (AAF/II) [1], the EAEC heat-stable enterotoxin 1 (EAST1) [9], and the cryptic secreted proteins Shf and AspU [8]. Chromosome associated markers include the Plasmid encoded toxin (Pet) [10], the protein involved in colonization (Pic) [8], and a

* Author for correspondence: Rua Botucatu 862, São Paulo, SP, 04023-062, Brazil.

Table 1. *PCR primers, cycles of amplification and sizes of amplified DNA fragments used as gene probes in this study*

Primer	Property	Sequence	Amplification cycle	Amplicon (bp)	Reference
aafC	Aggregative adherence fimbria (AAF/II) usher	5′-GTTATAGCTCGCACATATTC 3′-TGCAGACTGATAATGCTC	1 min 94 °C, 1 min 48 °C, 1 min 72 °C (30 cycles)	1144	This study
aggC	Aggregative adherence fimbria (AAF/I) usher	5′-TATTAAACCATGGTAGCG 3′-GCCAAGATCCGAGATTGA	1 min 94 °C, 1 min 45 °C, 1 min 72 °C (30 cycles)	538	[18]
aggR	Transcriptional activator of AAF/I and AAF/II	5′-CTAATTGTACAATCGATGTA 3′-ATGAAGTAATTCTTGAAT	1 min 94 °C, 1 min 40 °C, 1 min 72 °C (30 cycles)	308	[8]
aspU	Cryptic secreted protein	5′-CTTTTCTGGCATCTTGGGT 3′-GTAACAACCCCTTTGGAAGT	1 min 94 °C, 1 min 51 °C, 1 min 72 °C (30 cycles)	232	[8]
astA	EAEC heat-stable enterotoxin I (EAST1)	5′-CCATCAACACAGTATATCCGA 3′-GGTCGCGAGTGACGGCTTTGT	1 min 94 °C, 1 min 58 °C, 1 min 72 °C (30 cycles)	111	[19]
shf	Cryptic ORF	5′-ACTTTCTCCCGAGACATTC 3′-CTTTAGCGGGAGCATTCAT	1 min 94 °C, 1 min 51 °C, 1 min 72 °C (30 cycles)	613	[8]
irp2	Yersiniabactin biosynthesis gene	5′-AAGGATTCGCTGTTACCGGAC 3′-TCGTCGGGCAGCGTTTCTTCT	1 min 94 °C, 1 min 55 °C, 1 min 72 °C (30 cycles)	264	[8]
pet	Plasmid encoded toxin (Pet)	5′-GTGTTTCAACCAGGTTCAACA 3′-CCTTCACCAATTTTATGCAGT	1 min 94 °C, 1 min 52 °C, 90 sec 72 °C (30 cycles)	1037	[3]
pic	Protein involved in colonization (Pic)	5′-GGGTATTGTCCGTTCCGAT 3′-ACAACGATACCGTCTCCCG	1 min 94 °C, 1 min 52 °C, 90 sec 72 °C (30 cycles)	1175	[8]

Table 2. *Prevalence of individual putative virulence markers among EAEC strains from children with and without diarrhoea carrying and lacking the EAEC probe sequence*

	No. (%) of EAEC probe positive strains*			No. (%) of EAEC probe negative strains*		
Genes	Total	Cases† ($n = 31$)	Controls‡ ($n = 39$)	Total	Cases ($n = 31$)	Controls ($n = 27$)
aafA§	6	4 (12·9)	2 (5·1)	0	0	0
aafC	7	4 (12·9)	3 (7·7)	1	1 (3·2)	0
aggA§	15	7 (22·6)	8 (20·5)	0	0	0
aggC	20	8 (25·8)	12 (30·8)	0	0	0
aggR	52	25 (80·6)	27 (69·2)	1	1 (3·2)	0
aspU	66	29 (93·5)	37 (94·9)	9	4 (12·9)	5 (18·5)
astA	39	15 (48·4)	24 (61·5)	26	11 (35·5)	15 (55·6)
pet	16	9 (29·0)	7 (17·9)	0	0	0
shf	33	17 (54·8)	16 (41·0)	7	2 (6·5)	5 (18·5)
irp2	64	26 (83·9)	38 (97·4)	13	6 (19·4)	7 (25·9)
pic	35	15 (48·4)	20 (51·3)	2	1 (3·2)	1 (3·7)
None	0	0	0	24	16 (51·6)	8 (29·6)

* EAEC probe, reaction with CVD432 probe as determined by Gomes et al. [4].
† Cases, number of EAEC strains from children with diarrhoea.
‡ Controls, number of EAEC strains from children without diarrhoea.
§ Presence of *aggA* and *aafA* as determined by Elias et al. [12].

protein involved in yersiniabactin expression designated iron-repressible high-molecular-mass protein 2 (Irp2) [8, 11].

None of the EAEC markers is present in all EAEC strains [3, 8, 12–14], suggesting that EAEC is heterogeneous. However, it is not known whether such heterogeneity is related to the presence of the EAEC probe sequence and/or to variations in the AA phenotype. Moreover, few studies have compared the presence of all EAEC markers in strains isolated from children with and without diarrhoea in different geographic regions.

This study was conducted as an attempt to elucidate the heterogeneous nature of EAEC. For this purpose, the prevalence of EAEC markers in EAEC strains carrying and lacking the EAEC probe sequence and showing variations in the AA phenotype was analysed. In addition, the distribution of these markers in EAEC strains isolated from children with and without diarrhoea in São Paulo, Brazil, was determined.

MATERIALS AND METHODS

Origin and characteristics of the bacterial strains studied

The 128 strains analysed were identified as EAEC in a previous case-control study [4], by their reactivity with the EAEC probe [7] and/or pattern of adherence to HeLa cells [15]. That study was conducted with 200 children (1–4 years old) with acute diarrhoea (cases) and 200 non-diarrhoea (controls) who had visited the emergency room of Hospital Infantil Menino Jesus, which provides free medical assistance to children of low socio-economic status in the city of São Paulo, Brazil [4]. Among those children, 122 (60 cases and 62 controls) carried one or more EAEC isolates. Except for 6 children (2 cases and 4 controls) that carried 2 distinct types of EAEC, all other children carried only 1 type of EAEC isolate. Thus, a single representative isolate from each of the 116 children carrying only 1 type of EAEC, and 2 distinct isolates of the remaining 6 children were selected for the present study; each of these isolates was thus classified as a unique strain.

Among the 128 EAEC strains, 70 carried (EAEC probe$^+$) and 58 lacked (EAEC probe$^-$) the EAEC probe sequence. Regarding the variations in the AA phenotype, 53 strains of the EAEC probe$^+$ group presented AA, 7 AAcs and 10 were CD, while 27 strains of the EAEC probe- group showed AA and 31 AAcs [4]. The prevalence of genes encoding the structural subunits of AAF/I (*aggA*) and AAF/II (*aafA*) fimbriae was previously reported in this EAEC collection [12]. All strains lack virulence markers related to the other diarrheagenic *E. coli* categories,

https://doi.org/10.1017/S0950268802007136 Published online by Cambridge University Press

Table 3. *Combinations of putative virulence markers among EAEC strains reactive and non-reactive with the EAEC probe displaying the typical aggregative adherence pattern or variations of this pattern*

EAEC probe reaction* (No. of strains)	AA pattern (No. of strains)	Gene combinations†	No. (%) of strains
Positive (70)	AA‡ (53)	*irp2*	1 (1·9)
		aspU irp2	2 (3·8)
		aggR aspU irp2	4 (7·5)
		aspU astA irp2	6 (11·3)
		aspU shf irp2	1 (1·9)
		shf irp2 pic	1 (1·9)
		aggA aggC aggR aspU	1 (1·9)
		aggR aspU shf pic	1 (1·9)
		aggR aspU irp2 pic	2 (3·8)
		aspU astA shf irp2	2 (3·8)
		aggA aggC aggR aspU irp2	3 (5·7)
		aggC aggR aspU irp2 pic	1 (1·9)
		aggR aspU astA pet shf	1 (1·9)
		aggR aspU shf irp2 pic	3 (5·7)
		aspU astA shf irp2 pic	1 (1·9)
		aggA aggC aggR aspU astA irp2	1 (1·9)
		aggA aggC aggR aspU irp2 pic	1 (1·9)
		aggR aspU astA shf irp2 pic	1 (1·9)
		aggR aspU pet shf irp2 pic	1 (1·9)
		aggA aggC aggR aspU astA irp2 pic	3 (5·7)
		aggA aggC aggR aspU shf irp2 pic	1 (1·9)
		aggC aggR aspU astA shf irp2 pic	2 (3·8)
		aggR aspU astA pet shf irp pic	6 (11·3)
		aafA aafC aggR aspU astA pet shf irp2	1 (1·9)
		aafC aggR aspU astA pet shf irp2 pic	1 (1·9)
		aafA aafC aggR aspU astA pet shf irp2 pic	5 (9·4)
	AAcs§ (7)	*aspU irp2*	2 (28·6)
		aggR aspU irp2	1 (14·3)
		aggR astA irp2	1 (14·3)
		astA aspU irp2	1 (14·3)
		aggC aggR aspU astA irp2 pic	1 (14·3)
		aggR aspU astA pet shf irp2	1 (14·3)
	CD‖ (10)	*irp2*	1 (10·0)
		aggR aspU astA shf	1 (10·0)
		aggR aspU irp2 pic	1 (10·0)
		aggA aggC aggR aspU astA	1 (10·0)
		aggC aggR aspU irp2 pic	1 (10·0)
		aggR aspU shf irp2 pic	1 (10·0)
		aggA aggC aggR aspU astA shf	1 (10·0)
		aggA aggC aggR aspU astA irp2	1 (10·0)
		aggA aggC aggR aspU irp2 pic	1 (10·0)
		aggA aggC aggR aspU astA shf irp2	1 (10·0)
Negative (58)	AA (27)	None	10 (37·0)
		astA	7 (25·9)
		shf	1 (3·7)
		irp2	1 (3·7)
		astA irp2	3 (11·1)
		aspU irp2	1 (3·7)
		shf irp2	1 (3·7)
		astA pic	1 (3·7)
		aspU astA irp2	1 (3·7)
		aafC aggR aspU astA	1 (3·7)

https://doi.org/10.1017/S0950268802007136 Published online by Cambridge University Press

Table 3. (*cont.*)

EAEC probe reaction* (No. of strains)	AA pattern (No. of strains)	Gene combinations†	No. (%) of strains
	AAcs (31)	None	14 (45·2)
		astA	5 (16·1)
		irp2	3 (9·7)
		aspU astA	3 (9·7)
		astA shf	1 (3·2)
		shf irp2	1 (3·2)
		aspU astA irp2	1 (3·2)
		aspU astA shf	2 (6·4)
		astA shf irp2 pic	1 (3·2)

* EAEC probe, reaction with the CVD432 probe determined by Gomes et al. [4].
† *aggA* and *aafA* determined by Elias et al. [12].
‡ AA, typical aggregative adherence pattern to HeLa cells.
§ AAcs, aggregative adherence predominantly to the coverslip.
‖ CD, HeLa cell detachment.

i.e. enteropathogenic *E. coli* (*eae*, EAF and *bfpA*), enterotoxigenic *E. coli* (LT-I, LT-II, ST-Ip, ST-Ih and ST-II), enteroinvasive *E. coli* (Inv plasmid), and Shiga toxin-producing *E. coli* (*stx1* and *stx2*) [4]. The prototype EAEC strains 042 and 17-2 [8] were used as positive controls, and *E. coli* HB101 as a negative control.

Nucleic acid hybridization studies

The gene probes were obtained by PCR amplification using as template the genomic DNA of strains 042 (*aafC*, *aggR*, *aspU*, *shf*, *irp2*, *pet* and *pic* probes) and 17-2 (*aggC* and *astA* probes). The PCR primers, cycles of amplification and sizes of amplified DNA fragments are listed in Table 1. The *aafC* primers designed in this study were based on the *aafC* gene sequence published in GenBank (accession no. AF114828). DNA fragments were labelled by nick translation [16] using [α-^{32}P]dCTP, and used in colony blot assays performed under stringent conditions [17].

Statistical analysis

Statistical analyses were performed by Fisher's exact and χ^2 tests.

RESULTS

Table 2 shows the distribution of EAEC markers among 128 EAEC probe$^+$ and EAEC probe$^-$ strains. All gene sequences examined were most prevalent in the EAEC probe$^+$ group and moreover, all strains in this group had at least one EAEC marker. The most prevalent markers among the 70 EAEC probe$^+$ strains were *aspU* (66 strains), *irp2* (64 strains), and *aggR* (52 strains) whereas *astA* was most frequent among the strains of the EAEC probe$^-$ group (26 of 58 strains). Interestingly, 41·4% of all strains in this latter group carried none of the virulence markers examined. There were no significant statistical differences between the prevalence of any marker in cases and controls, in both EAEC probe$^+$ and probe$^-$ groups.

Table 3 shows the distribution of the EAEC markers among the strains, according to their reactivity with the EAEC probe and the variations in AA phenotype. Since there were no significant statistical differences in the prevalence of the different markers in strains from children with and without diarrhoea, in both EAEC probe groups, the combinations of markers displayed by the strains from cases and controls are presented altogether. The number of strains carrying two or more markers was higher in the EAEC probe$^+$ group (68 of 70 strains) than in the EAEC probe$^-$ group (17 of 58 strains). Consequently, the most complex gene combinations were found in the former group, regardless of the AA phenotype.

In the EAEC probe$^+$ group, strains harbouring 2 or more markers were found in 52 (98·1%) strains displaying AA, in all 7 (100%) strains displaying AAcs and in 9 (90%) strains displaying CD. Twenty-six different combinations of distinct EAEC markers were found among the AA, 6 combinations among the AAcs and 10 among the CD strains.

In the EAEC probe$^-$ group, most of the strains displayed only one or none of the markers. Moreover, strains lacking homology with any of the EAEC markers were found exclusively in this group. Strains presenting with 2 or more markers were found only in

https://doi.org/10.1017/S0950268802007136 Published online by Cambridge University Press

8 (29·6 %) strains displaying AA and 9 (29·0 %) strains showing AAcs. *astA* was the most prevalent gene sequence found in the AA (48·1 %) and AAcs (41·9 %) strains, followed by *irp2* (25·9 % and 19·3 %, respectively) and *aspU* (11·1 % and 19·3 %, respectively).

Notably, 5 strains (3 showing AA, 1 AAcs and 1 CD) that were devoid of *aggA* carried *aggC*. Likewise, 2 isolates displaying AA presented *aafC* but lacked *aafA*. These results suggest the presence of variants of AAF/I and AAF/II in these strains, respectively.

DISCUSSION

Various studies have reported on the heterogeneous prevalence of the different EAEC markers and demonstrated that their distribution may vary by location [3, 8, 12–14]. As an attempt to clarify the basis of this heterogeneity the prevalence of 11 putative EAEC virulence markers was investigated in a collection of EAEC strains isolated in São Paulo, Brazil. The properties screened included the presence of the EAEC probe sequence, variations in the AA phenotype, and their presence in children with and without diarrhoea.

In this study a correlation between a specific EAEC marker and diarrhoea was not found, even when the EAEC probe$^+$ and probe$^-$ strains were analysed separately. There are a few reports evaluating the prevalence of EAEC markers in strains isolated from case/control studies [12–14]. Except for the study conducted in Nigeria [13], where the presence of the gene related to the AAF/II fimbria was associated with diarrhoea, none of the EAEC markers has been statistically associated with this disease.

All EAEC markers studied here were more prevalent in the EAEC probe$^+$ group than in the EAEC probe$^-$ group. The most prevalent EAEC markers in the EAEC probe$^+$ group were *aspU* (94·3 %), *irp2* (91·4 %) and *aggR* (74·3 %), whereas in the EAEC probe$^-$ group were *astA* (44·8 %) and *irp2* (22·4 %). However, these two latter markers are not specific for the EAEC category, since *astA* has been found in other diarrhoeagenic *E. coli* categories [9] and *irp2* has been found in diffusely adhering *E. coli* and in different members of the *Enterobacteriaceae* [11, 20]. The *aspU*, *irp2*, *astA* and *aggR* markers have also been found in high prevalence in other populations studied [8, 11, 14], regardless of the presence of the EAEC probe sequence. Interestingly, among the strains isolated in Nigeria [13], the prevalence of *aspU* was much lower, whereas the prevalence of *aggR*, *pet* and *aggA* was much higher than those found in this and other studies [3, 8, 11, 14]. Such results might thus indicate the presence of a few clones distributed in that population.

As we have reported previously, the AAF/I and AAF/II structural genes (*aggA* and *aafA*, respectively) occurred in low prevalence in the EAEC strains examined here [12]. However, the occurrence of *aggC* and *aafC* (encoding the ushers of AAF/I and AAF/II, respectively) in strains devoid of the corresponding structural genes suggests that these strains produce AAF/I and AAF/II variant fimbriae. Occurrence of AAF/I variants but not AAF/II variants was previously reported [3, 18].

There are no reports evaluating the prevalence of the EAEC markers in relation to the variations of the AA pattern and the reactivity with the EAEC probe. In this study, a great variety of combinations of EAEC markers (34) was obtained among the EAEC probe$^+$ strains displaying AA, AAcs and CD. On the other hand, the strains displaying AA or AAcs of the EAEC probe$^-$ group, presented few gene combinations of EAEC markers [13]. No association with any of these AA variants and EAEC markers was observed. In another study where only *astA*, *aggA*, *aggC*, *aafA* and *pet* prevalences were investigated, a variety of combinations were also detected among EAEC probe$^+$ strains displaying AA and AAcel [3]. Okeke et al. [13] suggested that only EAEC strains presenting at least two putative EAEC virulence markers should be considered as potential pathogens. Taking this criterion in consideration, in our population, potentially pathogenic EAEC strains were found mainly among the EAEC probe$^+$ strains regardless of the AA phenotype.

In conclusion, our data clearly indicate that the EAEC strains isolated in a single location presents heterogeneous combinations of putative virulence genes. It was not possible to associate any of the EAEC markers (or combinations of these markers) with any of the variations of the AA phenotype displayed by the strains. Furthermore, our study demonstrates for the first time that the recognized EAEC heterogeneity may be correlated with the presence of the EAEC probe sequence. Therefore, the presence of this sequence could determine two EAEC subpopulations. Since our EAEC probe$^+$ strains were characterized by a higher number of virulence markers, these strains might represent a subpopulation with a higher pathogenic potential. Alternatively, EAEC probe$^-$ strains might contain additional viru-

lence factors so far not described. In order to elucidate these hypotheses, the pathogenic potential of EAEC probe$^-$ strains and a comparison of high molecular weight plasmids present in both groups are under investigation in our laboratories.

The fact that both EAEC probe groups were not statistically associated with acute diarrhoea could be due to the high level of asymptomatic carriage of strains of both groups. Lack of statistical association with this disease is often observed with other well-established enteropathogens in our population [21].

Whether the correlation between EAEC heterogeneity and presence of the EAEC probe sequence found in the present population is valid for other populations is unknown, since most epidemiological studies reported so far do not discriminate strains as EAEC probe positive and negative.

ACKNOWLEDGEMENTS

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (grants 94/1980-6 to T.A.T.G. and 00/05256-3 to L.R.T., and fellowships to W.P.E., A.P.U. and S.K.T.), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant 521160/98-7 to L.R.T.).

REFERENCES

1. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998; **11**: 142–201.
2. Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial P, Levine MM. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr Infect Dis J 1987; **6**: 829–31.
3. Gioppo NMR, Elias WP Jr, Vidotto MC, et al. Prevalence of HEp-2 cell-adherent *Escherichia coli* and characterisation of enteroaggregative *E. coli* and chain-like adherent *E. coli* isolated from children with and without diarrhoea, in Londrina, Brazil. FEMS Microbiol Lett 2000; **190**: 293–8.
4. Gomes TAT, Vieira MAM, Abe CM, Rodrigues D, Griffin PM, Ramos SRTS. Adherence patterns and adherence-related DNA sequences in *Escherichia coli* isolates from children with and without diarrhea in São Paulo city, Brazil. J Clin Microbiol 1998; **36**: 3609–13.
5. Knutton S, Shaw RK, Bhan MK, et al. Ability of enteroaggregative *Escherichia coli* strains to adhere in vitro to human intestinal mucosa. Infect Immun 1992; **60**: 2083–91.
6. Gomes TAT, Abe CM, Marques LRM. Detection of HeLa cell-detaching activity and alpha-hemolysin production in enteroaggregative *Escherichia coli* strains isolated from feces of Brazilian children. J Clin Microbiol 1995; **33**: 3364.
7. Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J Infect Dis 1990; **161**: 1249–51.
8. Czeczulin JR, Whittam TS, Henderson IR, Navarro-Garcia F, Nataro JP. Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. Infect Immun 1999; **67**: 2692–9.
9. Savarino SJ, Mcveigh A, Watson J, et al. Enteroaggregative *Escherichia coli* heat-stable enterotoxin is not restricted to enteroaggregative *E. coli*. J Infect Dis 1996; **173**: 1019–22.
10. Eslava C, Navarro-Garcia F, Czeczulin JR, Henderson IR, Cravioto A, Nataro JP. Pet, an autotransporter enterotoxin from enteroaggregative *Escherichia coli*. Infect Immun 1998; **66**: 3155–63.
11. Schubert S, Rakin A, Karch H, Carniel E, Heesemann J. Prevalence of the ''high-pathogenicity island'' of *Yersinia* species among *Escherichia coli* strains that are pathogenic to humans. Infect Immun 1998; **66**: 480–5.
12. Elias WP, Suzart S, Trabulsi LR, Nataro JP, Gomes TAT. Distribution of *aggA* and *aafA* gene sequences among *Escherichia coli* isolates with genotypic or phenotypic characteristics, or both, of enteroaggregative *E. coli*. J Med Microbiol 1999; **48**: 597–9.
13. Okeke IN, Lamikanra A, Czeczulin J, Dubovsky F, Kaper JB, Nataro JP. Heterogeneous virulence of enteroaggregative *Escherichia coli* strains isolated from children in southwest Nigeria. J Infect Dis 2000; **181**: 252–60.
14. Vila J, Vargas M, Henderson IR, Gascón J, Nataro JP. Enteroaggregative *Escherichia coli* virulence factors in traveler's diarrhea strains. J Infect Dis 2000; **182**: 1780–3.
15. Cravioto A, Gross RJ, Scotland SM, Rowe B. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr Microbiol 1979; **3**: 95–9.
16. Rigby PWJ, Dieckmann M, Rhodes C, Berg P. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 1977; **113**: 237–51.
17. Maas R. An improved colony hybridization method with significantly increased sensitivity for detection of single genes. Plasmid 1983; **10**: 296–8.
18. Rich C, Favre-Bonte S, Sapena F, Joly B, Forestier C. Characterization of enteroaggregative *Escherichia coli* isolates. FEMS Microbiol Lett 1999; **173**: 55–61.
19. Yamamoto T, Wakisaka N, Sato F, Kato A. Comparison of the nucleotide sequence of enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 genes among diarrhea-associated *Escherichia coli*. FEMS Microbiol Lett 1997; **147**: 89–95.
20. Bach S, Almeida A, Carniel E. The *Yersinia* high-pathogenicity island is present in different members of the family *Enterobacteriaceae*. FEMS Microbiol Lett 2000; **183**: 289–94.
21. Gomes TAT, Rassi V, MacDonald KL, et al. Enteropathogens associated with acute diarrheal disease in urban infants in São Paulo, Brazil. J Infect Dis 1991; **164**: 331–7.