Diarrheagenic *Escherichia coli* Markers and Phenotypes among Fecal *E. coli* Isolates Collected from Nicaraguan Infants[▽]

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We analyzed the prevalence of diarrheagenic *Escherichia coli* (DEC) markers and common phenotypes in
2,164 *E. coli* isolates from 282 DEC-positive samples. Enteropathogenic *E. coli* (EPEC) and enteroaggregative
E. coli (EAEC) were very diverse and were not correlated with diarrhea. Enterotoxigenic *E. coli* (ETEC) *estA*
and enterohemorrhagic *E. coli* (EHEC) belonged to a few phenotypes and were significantly correlated with
diarrhea.

In Nicaragua, diarrheagenic *Escherichia coli* (DEC) strains are considered to be common causes of diarrhea among infants, with enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) the most common DEC types found (4, 6, 12). We previously performed a large screening study on stool samples from 526 infants with and without diarrhea to detect the prevalence of five different categories of DEC and to determine the phenotypic diversity of *E. coli* isolates from diarrheal and control infants. A one-step multiplex PCR using a mixture of eight primer pairs was applied to the primary streak of *E. coli* cultures, and eight isolates per sample were analyzed by biochemical fingerprinting (7, 12). As many as 54% of the diarrheal samples and 53% of the control samples were positive for one or more DEC markers, and no clear connection between biochemical phenotypes found in DEC-positive samples and diarrhea could be established.

For the present report, we analyzed eight *E. coli* isolates from each of the 282 DEC–positive stool samples by PCR for the occurrence of the respective DEC markers found in the primary streak.

PCR and PhP typing of *E. coli* isolates. *E. coli* isolates analyzed in the study were recovered from a study conducted from March 2005 to September 2006 in León, Nicaragua, that examined 526 stool samples from children with ($n = 381$) and without ($n = 145$) diarrhea. All 282 samples that were positive for at least one of the DEC types, namely, enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC), according to a multiplex-PCR assay of the whole *E. coli* flora (12), were here further tested for the presence of DEC markers among eight randomly selected isolates per sample. Altogether, 2,164 *E. coli* colonies

(1,563 from diarrheal samples and 601 from controls) were examined for *eltB* and *estA* for ETEC, *eaeA* for EPEC, *vt1* and *vt2* for EHEC, *ial* for EIEC, and CVD432 for EAEC. PCR assays were performed using a 25-μl reaction mixture with a PuRe Taq Ready-To-Go PCR bead (GE Healthcare, United Kingdom) as previously described (12). The PCR products (10 μl) were electrophoresed on a 1.5% agarose gel and visualized under UV light after ethidium bromide staining. A 100-bp DNA ladder (Invitrogen Life Technologies) was used as a molecular mass marker. The same eight isolates per sample were previously typed using biochemical fingerprinting with PhP-RE plates of the PhenePlate system (PhPlate; http://www.phplate.se) (7) in order to define clonal groups and phenotypic diversity.

Student's *t* test was used to determine the statistical significance, where applicable.

DEC rates were calculated among the 282 DEC-positive samples as well as for all 4,009 isolates from all 526 previously studied samples (7) (Table 1). The EAEC (CVD432) marker was the most frequent and, surprisingly, seemed more common among isolates from controls than among isolates from diarrheal infants (Table 1). This observation is in agreement with other studies, where EAEC was also isolated from controls at frequencies equal to or higher than the rates seen with diarrheal children (1, 8, 11), indicating that such bacteria may be endemic in many parts of the world. In addition, the high phenotypic diversity shown among diarrheal as well as control isolates indicates that this group is very heterogeneous, as proposed in other studies (3, 5, 9), and it is probable that only certain strains carrying the CVD432 marker are diarrheagenic. However, such strains did not seem to be common in this study. ETEC (*eltB* and/or *estA*)-positive isolates were clearly correlated with diarrhea ($P < 0.05$), partly due to the presence of *estA*-positive isolates that were isolated only from patients with diarrhea (Table 1). The low phenotypic diversity among ETEC isolates carrying the *estA* marker was due to the fact that they were dominated by a single phenotype. The correlation between EPEC (*eae*)-positive isolates and diarrhea was

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TABLE 1. Occurrence of DEC markers and diversity indices among *E. coli* isolates

DEC type	Marker(s)	Diarrheal group						Control group					
		No. of:			% of isolates positive:		Di[c]	No. of:			% of isolates positive:		Di[c]
		Samples tested	Isolates tested	Positive isolates	Among tested isolates[a]	Among all isolates[b]		Samples tested	Isolates tested	Positive isolates	Among tested isolates[a]	Among all isolates[b]	
EAEC	pCVD342	106	797	312	39.1	10.8	0.96	48	375	150	40.0	13.5	0.93
EPEC	*eaeA*	61	457	126	27.6[d]	4.3	0.94	30	236	40	16.9[d]	3.6	0.93
ETEC	All	78	602	189	31.4[d]	6.5[d]	0.95	12	93	14	15.0[d]	1.3[d]	0.97
	eltB (only)	60	465	148	31.8[d]	5.1[d]	0.95	12	93	14	15.0[d]	1.3[d]	0.97
	eltB + *estA*	18	137	41	29.9	1.4	0.67	0	0	0	0	0	NA[e]
EHEC	*vt2*	8	57	18	31.6	0.6	0.68	0	0	0	0	0	NA
EIEC	*ial*	3	20	2	10.0	0.1	NA	2	0	0	0	0	NA
All[f]		205	1,563	632	40.4	21.8	0.96	77	601	196	32.6	17.6	0.95

[a] Calculated as percentages of positive isolates among samples that were positive for the actual DEC marker according to the results of the primary screening using multiplex PCR.
[b] Calculated as percentages of positive isolates among all 2,900 diarrheal and 1,109 control isolates collected from 526 Nicaraguan infants.
[c] Di, diversity indices calculated as the total level of diversity among all isolates positive for each DEC type.
[d] Fisher's exact test. $P = 0.001$ (diarrheal versus control isolates).
[e] NA, not applicable.
[f] Several samples and isolates were positive for more than one DEC marker.

less obvious (Table 1), and the diversity among isolates both from diarrhea patients and from controls was high. Also, other studies have shown that EPEC strains constitute a complex *E. coli* group whose members contain a diversity of virulence markers that make it difficult to determine which strains are truly pathogenic (2, 10). The few isolates positive for the EHEC (*vt2*) marker were found only in samples from diarrhea patients and showed low diversity, indicating that they could be members of virulent clonal groups.

Thus, our combined data from PCR detection of virulence markers and biochemical fingerprinting for detection of clonal groups have indicated that the diversity among Nicaraguan DEC-positive isolates was high for EAEC and EPEC, and those DEC types could not be significantly correlated with diarrhea. For ETEC and EHEC, the correlation to diarrhea was obvious, and, in addition, ETEC *estA* and EHEC isolates showed low levels of diversity due to the predominance of certain biochemical phenotypes. Thus, it seems that diarrhea was often caused not by the presence of any *E. coli* strain carrying a DEC marker but more probably by certain virulent DEC clones. This finding raises questions about the diagnostic value of the use of PCR to determine DEC virulence genes in stool samples without further analysis.

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