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Diversity of intestinal *Escherichia coli* populations in Nicaraguan children with and without diarrhoea

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Escherichia coli remains an important aetiological agent of infantile diarrhoea in Nicaragua. However, little is known about whether there is a high prevalence of endemic strains or whether infection is due to the epidemic spread of virulent clones. This study was undertaken to determine the diversity and distribution of clonal groups in a population of intestinal *E. coli* isolated from the faeces of children from León, Nicaragua, with (n=381) and without (n=145) diarrhoea, between March 2005 and September 2006. All samples had been screened previously for the presence of diarrhoeagenic *E. coli* (DEC) markers by multiplex PCR. From each sample, 8 *E. coli* colonies (where available) were analysed by biochemical fingerprinting (PhP-RE system), yielding a total of 4009 tested isolates. On average, three different biochemical phenotypes (BPTs) were found among the eight colonies analysed from each sample. The total diversity, measured as Simpson's diversity index (Di), was 0.97 among all 4009 isolates studied. Cluster analysis of data from all 4009 isolates revealed 24 common BPTs (identified in at least 1 % of the isolates) and 234 less common BPTs. Similar Di values were obtained among isolates from infants with and without diarrhoea, indicating that no widespread outbreak of DEC had occurred. Moreover, among samples that were positive for the DEC types enteroaggregative *E. coli*, enteropathogenic *E. coli* and enterotoxigenic *E. coli* (ETEC) carrying the e*ltB* gene, the diversities were almost as high as among non-DEC samples, whereas samples positive for ETEC carrying *estA*, enteroinvasive *E. coli* and enterohaemorrhagic *E. coli* showed lower diversities, indicating the prevalence of virulent clonal groups among these samples. The PhenePlate patterns of the 24 common BPTs identified here were compared with those obtained from *E. coli* isolated in a cohort infant study performed in 1991–1992 in the same area. Only 4 % of the isolates from the 1990s were similar to any of the common BPTs found in the present study.

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INTRODUCTION

Escherichia coli is a member of the family *Enterobacteriaceae* encountered as a normal inhabitant of the human gut microflora. The species *E. coli* comprises a versatile and extremely diverse group of organisms and, in principle, each individual may harbour their own composition of strains in their gut flora. However, some strains have acquired genes that convert them to pathogens (Kuhnert *et al.*, 2000; Nataro & Kaper, 1998), which may cause intestinal and extra-intestinal infection of humans and other mammals (Kaper *et al.*, 2004; Nataro & Kaper, 1998; Shpigel *et al.*, 2008). Such strains are referred to as pathogenic clones and, if spread among individuals, they may cause outbreaks of disease.

In Nicaragua, previous studies on diarrhoeal illness have pinpointed diarrhoeagenic *E. coli* (DEC), mainly belonging to the enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) pathotypes, as the most common bacterial causes of diarrhoea in infants. However, DECs have also been isolated frequently from healthy controls (Mayatepek *et al.*, 1993; Paniagua *et al.*, 1997; Vilchez *et al.*, 2009). In order to elucidate the role of various diarrhoeal pathogens,

Abbreviations: BPT, biochemical phenotype; DEC, diarrhoeagenic *Escherichia coli*; Di, diversity index; EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohaemorrhagic *Escherichia* coli; EIEC, enteroinvasive *Escherichia* coli; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; PhP, PhenePlate; UNAN, National Autonomous University of Nicaragua.

including *E. coli*, among infants in León, Nicaragua, faecal samples from 381 children with and 145 children without diarrhoea were collected from March 2005 to September 2006, and analysed for the presence of pathogens.

In this study, we analysed the population structure of *E. coli* by applying a typing system [biochemical fingerprinting using the PhenePlate (PhP) system] to eight *E. coli* isolates from each faecal sample. In particular, we wanted to determine whether any specific clonal groups of *E. coli* dominated in samples from infants with diarrhoea. We also investigated the diversity of the faecal *E. coli* flora in individual infants.

METHODS

Study population and clinical assessment. This study was conducted between March 2005 and September 2006 in León, the second largest city in Nicaragua. Five health-care centres, including the emergency paediatric ward of the main hospital, were involved in this study. All enrolled participants were children with (n=381) or without (n=145) diarrhoea, aged $\leqslant$60 months. A clinical examination was performed on those children who were suffering from acute diarrhoea, following the World Health Organization strategy recommendations for diarrhoea management (WHO, 2000) and adopted by the Nicaraguan health system (MINSA).

Clinical and epidemiological data were obtained from each child and registered through questionnaires. A diarrhoea case was defined as the occurrence of three or more loose, liquid or watery stools, or at least one bloody, loose stool, in the preceding 24 h period (WHO, 2000). Control subjects were healthy children without a history of diarrhoea for at least 1 month who were attending a health-care programme at health centres in the community. Ethics clearance for this study was approved by the Medical Bioethics Committee of the Faculty of Medical Sciences at the National Autonomous University of Nicaragua (UNAN), León, Nicaragua (registration no. 62).

Stool samples and primary microbiological procedures. Specimens were collected in plastic containers without preservatives and transported at 4 °C to the Department of Microbiology at UNAN, León, Nicaragua, on the day of collection, where primary microbiological examinations were performed. From hospitalized children, samples were collected $\leqslant$24 h after admission. Stools were processed and analysed within 4 h of collection by standard culture and identification methods (Gillespie & Hawkey, 2006). In brief, stools were plated on MacConkey agar following overnight incubation at 37 °C. From each plate showing suspected growth of *E. coli*, a full loop of *E. coli*-like colonies was suspended in brain heart infusion broth containing 15 % (v/v) glycerol, and aliquots were frozen at –70 °C. All samples were transported to the Karolinska Institutet, Stockholm, Sweden, where further characterization was performed, including screening for DEC virulence genes in the total faecal *E. coli* flora by multiplex PCR (Vilchez *et al.*, 2009) and typing of the isolates using biochemical fingerprinting.

Biochemical fingerprinting of *E. coli* isolates with the PhP-RE system. After thawing, the samples were subcultured on MacConkey agar plates with overnight incubation at 37 °C. From each cultured plate, eight colonies with *E. coli*-like morphology (where available)

Table 1. Population characteristics and total diversities of *E. coli* subpopulations

Children with and without diarrhoea						
Age (months)	**Diarrhoea group**		**Control group**		**All**	
	No. of isolates	**Total Di***	**No. of isolates**	**Total Di***	**No. of isolates**	**Total Di***
$\leqslant$1	57	0.859	193	0.947	250	0.947
2–6	645	0.958	345	0.960	990	0.964
7–12	753	0.973	138	0.952	891	0.972
13–60	1445	0.968	433	0.957	1878	0.967
All	2900	0.970	1109	0.966	4009	0.970
Children with diarrhoea						
	Hospitalized		**Not hospitalized**		**All**	
	No. of isolates	**Total Di***	**No. of isolates**	**Total Di***	**No. of isolates**	**Total Di***
Clinical features						
Watery stools	499	0.957	1641	0.971	2140	0.960
Loose stools	127	0.931	633	0.969	760	0.969
Mucus stools	113	0.890	372	0.966	485	0.966
Therapy received†						
ORS	108	0.954	2274	0.972	2382	0.972
IV fluids	518	0.937	0	NA	518	0.937
All cases	626	0.957	2274	0.972	2900	0.970

NA, Not applicable.

*Total Di denotes the Di values calculated from all isolates in a given population.

†Therapy of rehydration sought depending on the dehydration status or severity of diarrhoea: ORS, oral rehydration solution; IV fluids, intravenous rehydration.

were selected. A total of 4009 *E. coli* colonies (2900 from diarrhoeal and 1109 from control children; Table 1) were typed using biochemical fingerprinting (Kühn, 1985) using PhP-RE plates of the PhP system (PhPlate – http://www.phplate.se). The reagents used in the PhP-RE plates and the method of typing of *E. coli* isolates have been described previously (Achá *et al.*, 2004; Landgren *et al.*, 2005). In brief, each PhP-RE plate contains 8 parallel sets of 11 dehydrated reagents (carbohydrates and amino acids), which provide a high level of discrimination among *E. coli* strains. Growth medium containing a pH indicator (bromothymol blue) was added to the PhP plates, and eight *E. coli* colonies per plate were randomly picked and suspended into the first wells of each row of the plate. The homogenized bacterial suspensions were then transferred to the remaining wells of the same row, containing the various dehydrated reagents. Test results were read after 16, 40 and 64 h incubation at 37 °C using a desktop scanner (HP Scanjet 7400c XPA) with a transparency adaptor. The images generated by the scanner were converted to numerical absorbance data by PhPWIN software (PhPlate) After the last reading, the mean values from the readings of each well were calculated to give a biochemical fingerprint consisting of 11 numerical values for each isolate tested. The biochemical fingerprints of all isolates were compared pairwise with each other and the similarity between each pair was calculated as the correlation coefficient. The similarity matrix thus obtained was subjected to cluster analysis using the UPGMA (Sneath & Sokal, 1973). Isolates showing identical biochemical fingerprints were assigned to the same biochemical phenotype (BPT). When the PhPWIN software matched at least 1 % of the 4009 analysed isolates into the same BPT, a tentative common BPT was defined. BPTs found in less than 1 % of isolates were termed 'others' in this study. The diversity was calculated using Simpson's index of diversity (Hunter & Gaston, 1988), a relative measure of the distribution of isolates into different types. A high value (maximum 1) indicates an even distribution of the isolates into many different types, whereas a low value (minimum 0) indicates one or few dominating types in the population. All data handling, including calculations of similarities, cluster analysis and calculations of diversities, was performed using the PhPWIN software.

Statistical analysis. A non-parametric Mann–Whitney *U*-test was used for pairwise comparisons, and a χ^2 test and Kruskal–Wallis test were used for contingency analysis, where applicable.

RESULTS AND DISCUSSION

Epidemiological typing of *E. coli* bacteria is normally performed on a limited number of isolates, which are subject to molecular typing methods, often PFGE (Casarez *et al.*, 2007; Shaheen *et al.*, 2009). More traditional methods, such as serotyping, require access to a large set of reference sera, although many isolates remain untypable. In the present study, a typing system that is able to handle a large number of isolates was needed. The PhP system used in this study can easily handle studies involving several thousand isolates (Ahmed *et al.*, 2005; Landgren *et al.*, 2005), and the software included has been designed for analysis of this number of isolates. Moreover, this PhP typing system has been proven to have a high discriminatory power among *E. coli* isolates of environmental as well as human origin (Landgren *et al.*, 2005; Vilanova *et al.*, 2004) and to be able to measure stable characteristics of the *E. coli* flora (Achá *et al.*, 2005; Katouli *et al.*, 1990).

Total diversity of the *E. coli* populations

Previous studies performed on *E. coli* using the PhP system have generated a database containing PhP data for more than 30 000 *E. coli* isolates. These studies have shown that normal populations of *E. coli* of faecal origin (i.e. populations derived from non-outbreak situations) always show diversity index (Di) values above 0.96 (Achá *et al.*, 2004; Ahmed *et al.*, 2007; Vilanova *et al.*, 2004). In contrast, isolates derived from infections that may be caused by virulent clones of *E. coli* show lower diversities. As an example, a study on 2481 *E. coli* from lower urinary tract infections yielded a total diversity of 0.94 (Landgren *et al.*, 2005), and in other studies on ETEC from different sources, the isolates always indicated clusters of identical strains when subjected to PhP typing (Kühn *et al.*, 1985; Kühn & Möllby, 1986).

Thus, the total Di, as calculated in the present study, is a simple shortcut to answer the question of whether we often find the same type (strain) in different samples in a given population, indicating an epidemic outbreak among the studied samples. Table 1 shows the Di values of the *E. coli* populations studied as total Di (diversity among all isolates of each sample type). The total Di of all 4009 *E. coli* isolates was 0.97, which is similar to values that have been obtained in other studies on normal *E. coli* of faecal origin using PhP-RE plates (Achá *et al.*, 2004; Vilanova *et al.*, 2004). The Di values among isolates from various subpopulations were also calculated (Table 1), and it was found that in some cases values lower than 0.97 were obtained, indicating that different infants were colonized by the same strains more often than in a normal population. This was observed for example in diarrhoeal children of $\leqslant$1 month (0.859) (Table 1) and in hospitalized children with mucus stools (0.890). A slightly lower diversity (0.937) was observed among isolates from children who were attending the hospital and who received intravenous rehydration due to severe diarrhoea than among those children who visited a health-care centre in the community, possibly indicating either transmission and/or colonization with virulent *E. coli* clones that were capable of causing severe dehydration among this group, or that colonization by strains of hospital origin has occurred in some infants. Moreover, isolates from infants with diarrhoea who had received treatment with the antibiotics ampicillin and amoxicillin at least 1 month before the present diarrhoea episode showed low diversities (0.89 and 0.85, respectively; data not shown). This could be due to transmission of antibiotic-resistant clones among these infants as a selection pressure in favour of *E. coli* strains possessing genes priming for resistance.

Overall, however, isolates from diarrhoeal samples showed similar Di values compared with non-diarrhoeal samples, indicating that there was no clear dominance of certain pathogenic *E. coli* clones among these samples. Thus, there was probably no large outbreak of DEC clones during the study period (Table 1).

Intra-diversities of *E. coli* flora in stool samples

The eight colonies analysed for each sample yielded a mean of three BPTs per sample. The diversities ranged between 0 (all isolates identical) and 1.0 (all isolates belonged to different BPTs) for each sample. Fig. 1 exemplifies the varying diversities in dendrograms derived from cluster analysis of *E. coli* isolates from six faecal samples, in which the BPT distributions are shown for high, medium and low diversities. There was a clear tendency, albeit non-significant (P=0.088, Kruskal–Wallis non-parametric test), towards a lower intra-diversity in general in samples from diarrhoeal children compared with those from controls. Lower diversity would be expected if the diarrhoea was caused by overgrowth of a pathogenic *E. coli* clone. However, it could also be a 'wash-out effect' caused by the loose stools that eliminate transient *E. coli* strains in the normal flora. Intra-diversity differed significantly between ages (P=0.030; Table 2). Pair-wise comparisons yielded significant increases in intra-diversities, for example between children aged 0–6 months versus the older groups (P=0.04 and P=0.024, respectively), indicating a less mature flora among the younger children (Alm *et al.*, 2002). This was especially pronounced among infants aged $\leqslant$1 month. Probably, the lower aged infants have not yet been exposed to many different *E. coli* strains and are therefore normally colonized by fewer *E. coli* strains (Adlerberth *et al.*, 1998).

The intestinal *E. coli* flora in humans and other mammals normally contains many different *E. coli* clones, of which a few are residential (i.e. persistent over time), whereas the majority are only occasional visitors that may be found in the gut microflora on a single occasion (Adlerberth *et al.*, 1998; Sears *et al.*, 1950) or on a few occasions closely spaced in time after they have been ingested via food or water (Duriez *et al.*, 2001; Gorter *et al.*, 1998). However, the number of *E. coli* types detected in the gut flora increases with the number of isolates analysed. For example, Zoric *et al.* (2002) analysed 144 *E. coli* isolates from each of 6 healthy pigs using PhP typing and found up to 48 different BPTs per pig (Zoric *et al.*, 2002). Thus, it is possible that important strains may escape detection, even though many colonies per sample are analysed, and searching for DEC-positive stool samples by analysing just one or two single colonies per sample would probably result in many false negatives. For the samples in the present study, PCR for DEC was performed on the total *E. coli* flora (Vilchez *et al.*, 2009), which should yield a higher rate of DEC-positive samples than if performed on single colonies.

Besides low age, the factor that seemed mainly to be correlated with a low diversity of intestinal *E. coli* flora in

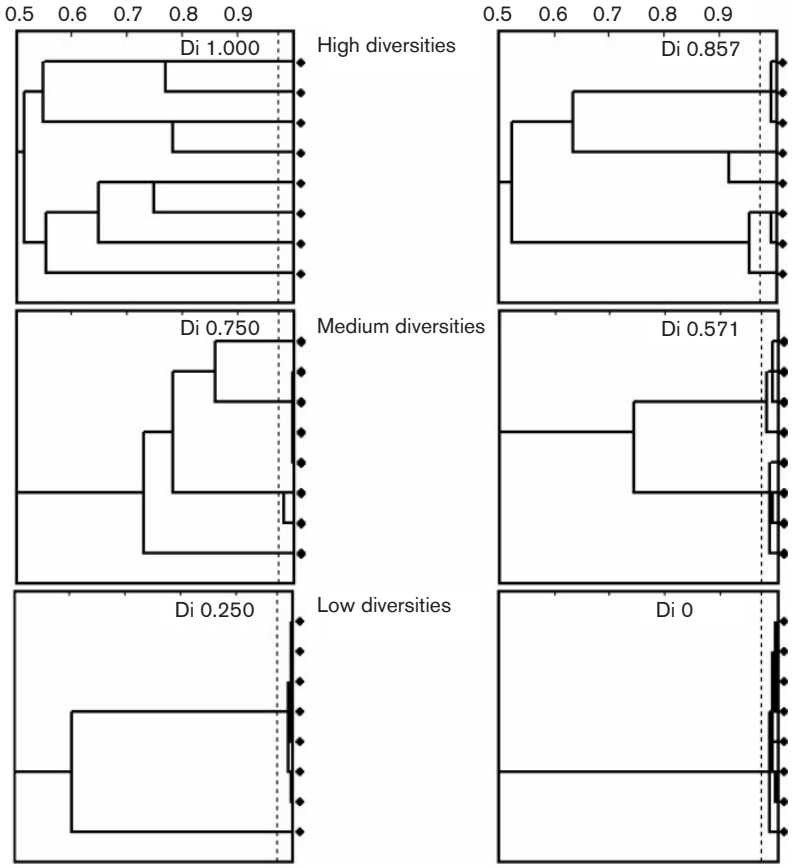

Fig. 1. Examples of dendrograms derived from UPGMA clustering of *E. coli* isolates from six faecal samples showing various degrees of intra-diversity among the eight isolates typed. Vertical dotted lines denote the pre-defined identity level.

Table 2. Intra-diversities of *E. coli* expressed as mean Di within individual samples

Values with different superscript letters following them have significant differences between the compared age groups ($P<0.05$).

Children with and without diarrhoea						
Age (months)	**Diarrhoea group**		**Control group**		**All**	
	No. of samples	**Intra-Di* (mean ± SD)**	**No. of samples**	**Intra-Di* (mean ± SD)**	**No. of samples**	**Intra-Di* (mean ± SD)**
⩽1	8	0.326 ± 0.391	25	0.405 ± 0.330[c]	33	0.386 ± 0.341
2–6	85	0.379 ± 0.320[a,b]	45	0.487 ± 0.335	130	0.417 ± 0.328[d,e]
7–12	99	0.481 ± 0.355[a]	18	0.626 ± 0.314[c]	117	0.503 ± 0.352[d]
13–60	189	0.480 ± 0.332[b]	57	0.532 ± 0.386	246	0.492 ± 0.345[e]
All	381	0.455 ± 0.339	145	0.508 ± 0.355	526	0.469 ± 0.344
Children with diarrhoea						
	Hospitalized		**Not hospitalized**		**All**	
	No. of samples	**Intra-Di* (mean ± SD)**	**No. of samples**	**Intra-Di* (mean ± SD)**	**No. of samples**	**Intra-Di* (mean ± SD)**
Clinical features						
Watery stools	67	0.410 ± 0.358	214	0.452 ± 0.329	281	0.442 ± 0.336
Loose stools	16	0.536 ± 0.254	84	0.480 ± 0.360	100	0.484 ± 0.345
Mucus stools	15	0.503 ± 0.334	50	0.484 ± 0.350	65	0.489 ± 0.344
Therapy received†						
ORS	15	0.547 ± 0.371	298	0.460 ± 0.338	313	0.464 ± 0.339
IV fluids	68	0.410 ± 0.334	0	NA	68	0.410 ± 0.334
All cases	83	0.434 ± 0.343	298	0.460 ± 0.338	381	0.455 ± 0.339

*Intra-Di denotes the mean Di value calculated from the eight isolates in individual samples.
†Therapy of rehydration sought depending on the dehydration status or severity of diarrhoea: ORS, oral rehydration solution; IV fluids, intravenous rehydration.

Table 3. Occurrence of virulence markers among *E. coli* flora from children with and without diarrhoea in relation to diversities of *E. coli* isolates

DEC type	**Target gene**	**Population**							
		Diarrhoea group (*n*=381)				**Control group (*n*=145)**			
		No. (%) of:*		**Total Di†**	**Intra-Di‡**	**No. (%) of:***		**Total Di†**	**Intra-Di‡**
		Samples	**Isolates**			**Samples**	**Isolates**		
EAEC	*pCVD432*	106 (27.8)	797	0.958	0.506	48 (33.1)	375	0.952	0.679
EPEC	*eaeA*	61 (16.0)	457	0.938	0.607	30 (20.7)	236	0.946	0.625
EIEC	*ial*	3 (0.8)	20	0.821	0.476	2 (1.4)	13	0.833	0.497
ETEC	*eltB*	60 (15.7)	465	0.952	0.554	12 (8.3)	93	0.964	0.929
	estA	4 (1.0)	32	0.873	0.607	0 (0.0)	–	–	–
	eltB + *estA*	14 (3.7)	105	0.912	0.250	0 (0.0)	–	–	–
EHEC	*vt1*, *vt2*	8 (2.1)	57	0.829	0.560	0 (0.0)	–	–	–
Non-DEC		176 (46.2)	1337	0.959	0.464	68 (46.9)	508	0.953	0.429

*The total percentage exceeds 100, as many of the children [48 (12.6 %) with diarrhoea and 15 (10.3 %) controls] were positive for more than one DEC type.
†Total Di denotes the Di values calculated among all isolates belonging to each sample type.
‡Intra-Di denotes the mean value of the Di values calculated for the eight isolates from each individual sample.

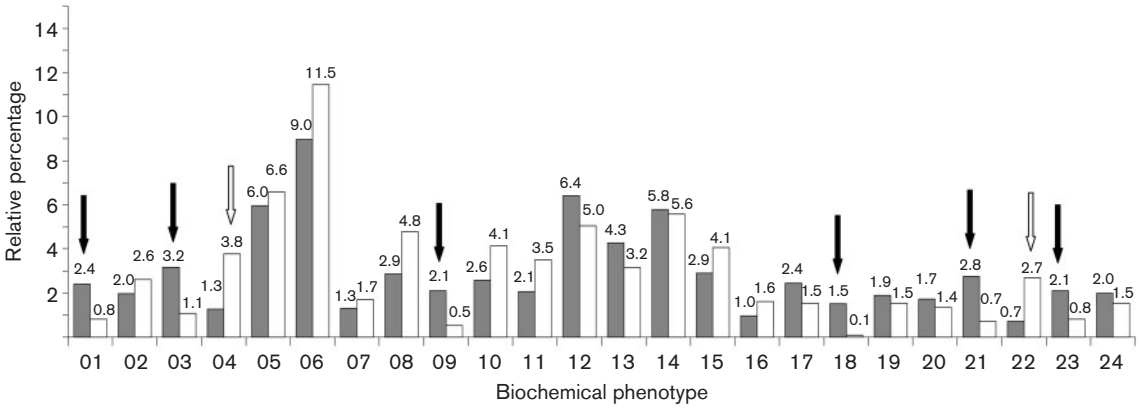

Fig. 2. Relative distribution of the most common BPTs of *E. coli* found among the studied population, calculated as percentages of the total number of isolates within each group (i.e. diarrhoeal and control). Black arrows indicate BPTs that were found predominantly in children with diarrhoea, and white arrows indicate BPTs that were found predominantly in control children. These BPTs were significantly different between isolates originating from diarrhoeal patients and those of controls ($P<0.01$). Grey bars, diarrhoeal group ($n=2900$); white bars, control group ($n=1109$).

individual children was the presence of ETEC carrying the *estA* gene (Table 3). ETEC with *estA* were isolated only from infants with diarrhoea, and both the low total diversities among all isolates from the 18 positive children and the intra-sample diversities indicated that colonization with a limited number of pathogenic clones may have caused the diarrhoea in these children.

Common BPTs

Cluster analysis of data from all 4009 isolates revealed 24 common BPTs comprising 70 % of the isolates, and 234 other BPTs found in only one or a few infants, comprising 30 % of the isolates. Fig. 2 shows the relative distribution of the common BPTs among the *E. coli* isolates from children with and without diarrhoea. Most BPTs seemed to be distributed equally among the diarrhoeal and control children. However, some BPTs (e.g. 01, 03, 09, 18, 21 and 23) were significantly more common among children with diarrhoea than in children without diarrhoea, whereas certain BPTs (e.g. 04 and 22) were more common among the control children. The most common type found in the present study was BPT06, comprising almost 387 isolates (9.7 %). The biochemical fingerprint of this BPT could represent a clonal group of *E. coli* that is particularly common in the area of León, but it could also represent a particularly common *E. coli* fermentation pattern that is found in many clonal groups. Therefore, we compared the

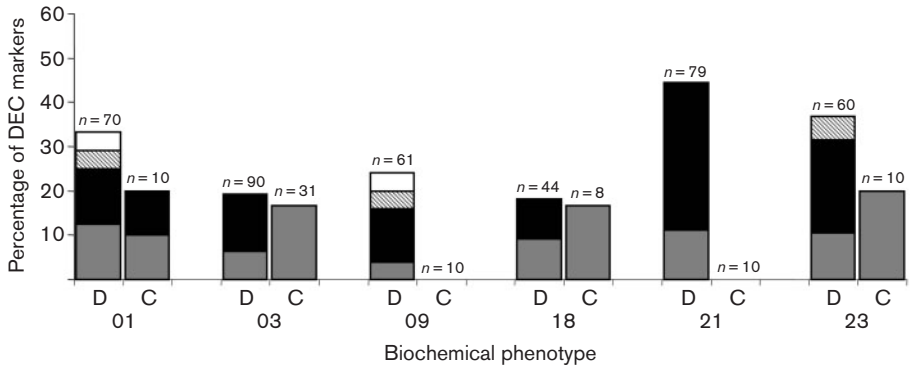

Fig. 3. BPTs of *E. coli* found predominantly in children with diarrhoea (indicated with black arrows in Fig. 2) and the proportion of samples presenting predominant DEC markers in these BPTs. The figure shows that *eltB*+*estA*-positive samples occurred mainly in BPTs 01 and 09 (*eltB*, heat-labile toxin gene; *estA*, heat-stable toxin gene). Furthermore, it can be seen that not only were these BPTs found more often in diarrhoeal children (Fig. 2), but those from diarrhoeal samples were more often associated with virulence factors detected in the samples, especially types 09 and 21. D, Diarrhoeal group; C, control group. Grey bars, EPEC (*eaeA* – attaching and effacing gene); black bars, ETEC (*eltB*); hatched bars, ETEC (*estA*); white bars, ETEC (*eltB*+*estA*).

biochemical fingerprint of this BPT with those obtained from 4900 isolates collected during other studies (Alm *et al.*, 2002; unpublished data) on faecal flora of infants. Among these isolates, less than 4 % matched BPT06 (data not shown), and thus the high prevalence of this BPT seems to mainly reflect an endemic origin in this studied population.

In 1991–1992, a cohort study on *E. coli* diarrhoea in children from León was performed (Paniagua *et al.*, 1997) and 797 isolates from that study were also subjected to PhP typing. The PhP patterns of those isolates were compared with the 24 common BPTs identified in the present study. Only 4 % of the isolates from 1991–1992 were similar to any of the common BPTs found in 2005–2006, and only one, BPT01, was found in more than 1 % of the isolates from 1991–1992 (unpublished data). This clearly illustrates that the *E. coli* population structure is changing continuously over time, which may be an important consideration for the development and evaluation of vaccines directed at protection against DEC infections.

Total diversities of *E. coli* flora among samples presenting DEC virulence genes

The total *E. coli* flora in all 526 stool samples studied had also been screened previously for DEC pathotypes by multiplex PCR (Vilchez *et al.*, 2009). In order to study whether DEC-positive samples might contain certain clonal groups of *E. coli*, the diversities among the *E. coli* isolates in these samples were calculated separately (Table 3). Enteroaggregative *E. coli* (EAEC) was the most common pathotype, and the *E. coli* isolates from EAEC-positive samples showed the same diversity as isolates from non-DEC samples. EAEC also showed a similar prevalence among diarrhoeal and control children. These findings reflect the heterogeneous nature of this *E. coli* type, as reported elsewhere (Nataro *et al.*, 1995; Suzart *et al.*, 2001). Hence, further characterization would be required to detect truly virulent EAEC strains.

Isolates from samples positive for EPEC *eaeA* and ETEC *eltB* showed somewhat decreased diversities, whereas the low diversities among isolates from samples positive for ETEC *estA* were a clear indication that a limited number of BPTs were carriers of these virulence markers. These BPTs could represent pathogenic clonal groups that have spread among the infants (Table 3, Fig. 3). In addition, isolates from samples positive for enterohaemorrhagic *E. coli* (EHEC) and enteroinvasive *E. coli* (EIEC) showed low diversities, also indicating the presence of pathogenic clones. However, the number of samples containing these DEC types was rather small (Table 3).

In Fig. 4, an example of clustered PhP data is shown. The figure shows all 57 isolates from the 8 samples that were EHEC positive with the multiplex PCR screening. The dendrogram contains a cluster of 25 identical or similar isolates, derived from 6 out of 8 EHEC-positive samples, which might represent a clonal group of EHEC in this population.

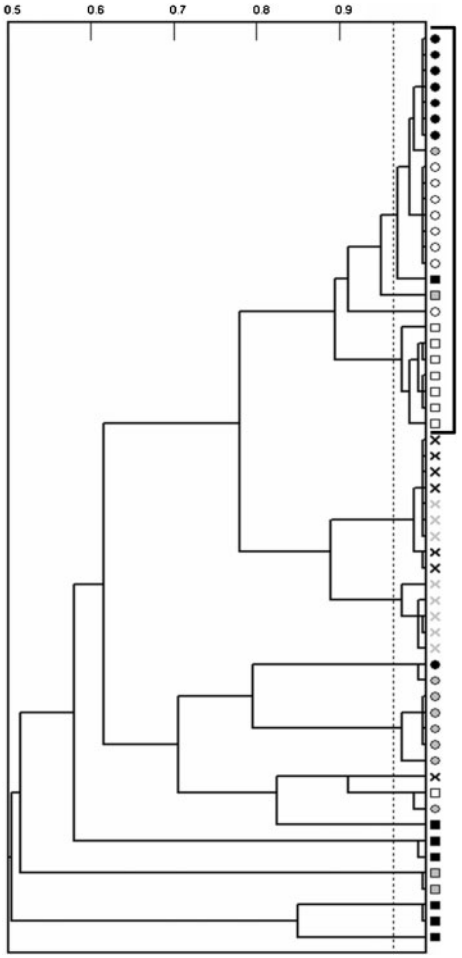

Fig. 4. Dendrogram depicting 57 *E. coli* isolates from 8 infant stool samples that were EHEC positive by multiplex PCR screening. The eight different symbols indicate isolates from the eight samples. The total diversity among the isolates was 0.829. The figure shows a cluster of identical or similar isolates (vertical solid line) from six different infants. These isolates could represent a virulent clonal group.

Thus, the total diversities and distribution of BPTs found in the present study give no indication that diarrhoea in infants in León during 2005–2006 was due to any large outbreaks of pathogenic *E. coli*, although a large number of faecal samples contained DECs. The high prevalence of DEC-positive samples among both healthy controls and diarrhoeal infants could be due to a high endemic prevalence of many different DEC clones in this area that has resulted in a high rate of asymptomatic carriers. The fact that a significantly high proportion of infants with diarrhoea were colonized simultaneously with several DECs (Vilchez *et al.*, 2009) supports the suggestion that the infections could have been due to ingestion of contaminated food or water (Duriez *et al.*, 2001; Gorter *et al.*, 1998), rather than a result of horizontal transmission between individuals.

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