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Enteropathogenic and enteroaggregative *E. coli* in stools of children with acute gastroenteritis in Davidson County, Tennessee

Monique A. Foster[a], **Junaid Iqbal**[a], **Chengxian Zhang**[a], **Rendie McHenry**[a], **Brent E. Cleveland**[a], **Yesenia Romero-Herazo**[a], **Chris Fonnesbeck**[a], **Daniel C. Payne**[b], **James D. Chappell**[a], **Natasha Halasa**[a], and **Oscar G. Gómez-Duarte**[a,*]

[a] Vanderbilt University School of Medicine, Nashville, TN

[b] Centers for Disease Control and Prevention, Atlanta, GA

Abstract

This prospective acute gastroenteritis (AGE) surveillance was conducted in the inpatient and emergency room settings at a referral pediatric hospital to determine the prevalence of diarrheagenic *Escherichia coli* (DEC) in children<12 years of age with AGE in Davidson County, Tennessee. Subjects 15 days to 11 years of age, who presented with diarrhea and/or vomiting, were enrolled. Stool specimens were processed for detection of DEC using multiplex polymerase chain reaction. From December 1, 2011, to June 30, 2012, a total of 79 (38%) out of 206 stool specimens from children with AGE tested positive for *E. coli*. A total of 12 (5.8%) out of 206 stool specimens from children with AGE were positive for a DEC. Eight (67%) out of these 12 were positive for enteropathogenic *E. coli*, and the remaining 4 were positive for enteroaggregative *E. coli*. DEC clinical isolates clustered with known *E. coli* enteropathogens according to multilocus sequencing typing.

Keywords

E. coli; diarrhea; children; gastroenteritis; EPEC; EAEC; epidemiology

1. Introduction

The Centers for Disease Control and Prevention (CDC) estimates that 0.6 episodes of diarrhea per person occur every year in the United States (Jones et al., 2007). Rates are higher for children under the age of 5 years. CDC estimates that 1 in 6 Americans per year (or 48 million people) is infected by foodborne pathogens, resulting in 128,000 hospitalizations and 3000 deaths (Scallan et al., 2011a, 2011b). Most acute gastroenteritis (AGE) episodes (90%) were caused by 31 enteric pathogens including viruses 59%, bacteria (39%), and parasites (2%). The leading causes of AGE were norovirus (58%), nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (11%), *Campylobacter* spp. (9%), and *Staphylococcus aureus* (Scallan et al., 2011b). The leading causes of hospitalization were

* Corresponding author. Tel.: +1-615-343-5619; fax: +1-615-343-7659. Oscar.gomez@vanderbilt.edu (O.G. Gómez-Duarte)..

nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and Shiga toxin–producing *Escherichia coli* 0157, while the leading causes of death were nontyphoidal *Salmonella* spp. (28%), norovirus (11%), and *Campylobacter* spp. (6%).

Shiga toxin–producing *E. coli* (STEC) are an important cause of foodborne illnesses in the United States, and the severity of the infection results in hospitalization and death. STEC are leading causes of dysenteric diarrhea and mortality secondary to hemolytic uremic syndrome (HUS) (Bavaro, 2012; Johnson et al., 2006; Rangel et al., 2005). Limited information is available on other *E. coli* intestinal pathotypes. Based on their mechanism of pathogenicity and unique arrays of virulence factors, the different pathotypes are designated enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), diffusely adherent (DAEC), and Shiga toxin–producing (STEC)/ enterohemorrhagic *E. coli*. Since diagnostic tools to differentiate these *E. coli* pathogens are typically not available at clinical laboratories or reference laboratory centers, little is known about their epidemiology.

We attempted to characterize and describe the epidemiology of *E. coli* pathogens in children who presented with AGE at a large, academic medical center in Nashville, Tennessee. We isolated *E. coli* strains from stools of children with diarrhea in a defined geographic area and tested them for genetic markers of known pathotypes of diarrheagenic *E. coli* (DEC).

2. Materials and methods

2.1. Study design and enrollment

This prospective, cross-sectional active surveillance study enrolled children who were 15 days to 11 years old and who resided in Davidson County, Tennessee, presenting to the Vanderbilt Children's Hospital Emergency Department and inpatient units with diarrhea and/or vomiting from December 1, 2011, through June 30, 2012. AGE cases were enrolled if diarrhea, defined as ≥3 loose stools in a 24-hour period, or vomiting, ≥1 episode in a 24-hour period, had occurred. Subjects were excluded for the following criteria: not residing in Davidson County, Tennessee, having symptoms at the time of presentation lasting ≥10 days in duration, immunocompromised, or an inability to understand English or Spanish. Demographic data, illness characteristics, medication, and travel history were obtained by chart review and standardized questionnaires. This study was approved by the Vanderbilt University Institutional Review Board (no. 120099).

2.2. Processing of stool specimens

Specimens consisted of at least 1 gram of whole stool in sterile containers or swabs of stool preserved in Cary–Blair transport media. Specimens plated on MacConkey agar (Becton Dickinson and Company, Sparks, MD, USA) and incubated overnight at 37 °C were examined for the presence of lactose-fermenting, nonmucoid colonies. Such isolates were subcultured onto eosin–methylene blue (EMB) agar (Remel, Lenexa, KS, USA) and incubated for 18–24 hours at 37 °C. Colonies with characteristic *E. coli* morphology on EMB agar (metallic green sheen) were inoculated into sulfide-indole-motility medium (Neogen Corporation, Lansing, MI, USA) for biochemical testing. Presumptive *E. coli*

isolates that were indole positive, motile, gas-producing, and negative for hydrogen sulfide production were confirmed as *E. coli* and stored at −80 °C for further testing as described before (Gómez-Duarte et al., 2010).

2.3. Preparation of DNA and DNA amplification by polymerase chain reaction (PCR) assays

Genomic DNA was isolated from *E. coli* clinical isolates by culturing strains in 2-mL Luria broth (LB) at 37 °C with shaking at 225 rpm overnight. A 200-μL aliquot of bacterial culture was centrifuged and pellet resuspended in 500 μL of Tris-EDTA buffer, boiled for 5 minutes and centrifuged at 10,000×g for 3 minutes. The supernatant containing crude genomic DNA was used as template for PCR. Two separate multiplex PCR reactions were performed on each DNA sample as described before (Gómez-Duarte et al., 2010). Mix 1 detected EPEC, EAEC, and STEC, and mix 2 detected ETEC, EIEC, and DAEC. Both mix 1 and mix 2 reactions contained 18-μL PCR blue master mix (Invitrogen, Carlsbad, CA, USA), 1 μL of oligonucleotide mix 1 or oligonucleotide mix 2, and 1-μL DNA template. Reactions were preheated at 94 °C for 2 minutes and amplified for 40 cycles consisting of 30 seconds at 92 °C, 30 seconds at 58 °C, and 30 seconds at 72 °C. PCR products were separated by electrophoresis using a 2% agarose gel containing ethidium bromide. Gel images were captured and analyzed.

2.4. Serotyping

O– and H– typing of DEC isolates was conducted at Pennsylvania State *E. coli* Reference Center (Pennsylvania State University, University Park, PA, USA). O– serotyping was conducted using antisera generated against *E. coli* serogroups designated O1-O187 with the exceptions of O31, O47, O67, O72, O94, and O122 as these are not designated. H– typing was performed by PCR–restriction fragment length polymorphism of *fliC* flagellar gene responsible for H types.

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility to 12 different antibiotics was tested using BD BBL™ Sensi-Disc™ Susceptibility Test Discs methods (Becton, Dickinson and Company Sparks, MD, USA). Strain activity was tested against cefazolin, ceftriaxone, ampicillin, amoxicillin/clavulanic acid, ceftazidime, cefuroxime, cefepime, ciprofloxacin, gentamicin, meropenem, trimethoprim/sulfamethoxazole, and piperacillin/tazobactam (Qin et al., 2008). The zones of bacterial growth inhibition were interpreted according to the 2014 guidelines of the Clinical and Laboratory Standards Institute. Controls used for testing included the *E. coli* ATCC 29522 as negative control (susceptible to all antibiotics) and *Klebsiella pneumoniae* ATCC 700603D-5 as positive control (resistant to all beta-lactam antibiotics).

2.6. Biofilm formation assay

Biofilm quantitative assay was performed as described previously (Wakimoto et al., 2004). In brief, cultures were diluted in 297-μL Dulbecco's Modified Eagle Medium at a ratio of 1:100 in a microcentrifuge tube and vortexed. One hundred microliters of the bacteria dilution was added to a 96-well plate, which was sealed with an adhesive film. The plate was then incubated at 37 °C for 24 hours. The plate was washed, and 125 μL of 0.1% crystal

violet solution was added to each well and incubated at room temperature for 15 minutes. The crystal violet was discarded and plate rewashed and air dried. Two hundred microliters of 95% ethanol was added to each well and incubated at room temperature for 15 minutes. The solubilized crystal violet was transferred to a 96-well Immulon 2 HB plate, and the optical density (OD) was measured at 590 nm. A strain was considered positive if the OD ≥0.089.

2.7. Multilocus sequence typing (MLST)

E. coli clinical isolates were analyzed by MLST as described online (http://mlst.warwick.ac.uk/mlst/). Internal fragments from 7 housekeeping genes, *adk* (adenylate kinase), *fumC* (fumarate hydratase), *gyrB* (DNA gyrase), *icd* (isocitrate/isopropylmalate dehydrogenase), *mdh* (malate dehydrogenase), *purA* (adenylosuccinate dehydrogenase), and *recA* (ATP/GTP binding motif of recombinase A), were amplified by PCR and DNA sequenced as described before (Wirth et al., 2006). Sanger DNA sequencing was conducted by the DNA core facility at Vanderbilt University. Forward and reverse DNA sequences for each gene and strain were aligned for comparison and editing. Sequence editing was conducted with DNADynamo software (Blue Tractor Software, North Wales, UK). Sequences for the 7 genes of each strain were concatenated to produce an alignment sequence of 3423 bp. Alignment of concatamers used ClustalW software available online at http://www.phylogeny.fr/version2_cgi/simple_phylogeny.cgi (Dereeper et al., 2008, 2010). Phylogenetic trees for all *E. coli* isolate and *E. coli* control MLST concatamers were conducted by bootstrapping procedure. *E. coli* control MLSTs included STEC, EPEC and ETEC strains reported in the http://mlst.warwick.ac.uk/mlst/ database and previously isolated from human cases of diarrhea. Control *E. coli* ancestral phylogenetic groups sequences were also obtained from the same database, including the *E. coli* Reference (ECOR) collection (Ochman and Selander, 1984). MLST-based clonal groups were defined as 2 or more *E. coli* strains with identical MLST sequence.

2.8. Statistical analysis

Data were analyzed using descriptive statistics, and epidemiologic variable distributions were identified. Central tendency and dispersion values were calculated for all variables analyzed using STATA version 12.

3. Results

3.1. Demographic characteristics of children with diarrhea

During the study period, 410 subjects were enrolled with AGE, and 299 of them consented for further *E. coli* testing. From the 299 subjects enrolled, 206 (69%) provided stool specimens for further analysis (Table 1). Eighty percent of these subjects (165 of 206) were <5 years old, and 54% were <1 year old, and gender was similar (54% male, 46% female).

3.2. Diarrheagenic E. coli identified among children with diarrhea

E. coli isolates were recovered from 38% (79 of 206) of the AGE specimens. Five individual *E. coli* isolates were obtained from each stool specimen up. Six percent (12 of 206) of the AGE specimens were positive for DEC. In those specimens, a minimum of 1 out the

maximum of 5 *E. coli* isolates per stool specimen was positive. Of the 12 subjects positive for DEC, the distribution of pathotypes revealed EPEC (67%, n = 8) and EAEC (33%, n = 4). No STEC, ETEC, EIEC, or DAEC were identified. The mean age of subjects was 36 months (Table 2). The mean age of subjects positive for EPEC was 25 months (SD 38, range 2–114). Most cases of EPEC (6/8, 75%) were in children less than 5 years of age. The mean age of subjects positive for EAEC was 16.6 months (SD 16, range 5–39). All EAEC cases (4/4, 100%) were found in children less than 5 years of age (Table 2). None of the study subjects was found to have coinfections with multiple DEC. The majority of DEC were isolated from cases occurring during summer months.

3.3. Phenotypic and genotypic features of DEC strains

DEC isolates were characterized at the genetic and phenotypic levels. All EPEC strains (8 of 8) were positive for the *eae* gene and negative for the *bfpA* gene indicating that they were atypical EPEC (aEPEC). Diverse numbers of serotypes were associated with EPEC strains, which included 4 O groups and 5 H groups. The majority of EPEC strains, 62% (5 of 8), were resistant to ampicillin (Table 3). All EAEC clinical isolates were positive for *aggR* and for *aaiC*. Three different O and H groups were recognized in the 4 EAEC clinical isolates. Only 2 strains had an identical serotype. All EAEC were resistant to ampicillin.

3.4. Few EAEC clinical isolates form biofilm of inert surfaces

EAEC strains typically form biofilms over inert and biological surfaces. To evaluate if EAEC clinical isolates form biofilms, individual colonies from cases with AGE were tested in a biofilm assay. Only 2 EAEC isolates from a single subject (ENIE-0245) were positive for biofilm formation (Fig. 1). The remaining EAEC isolates from the same subject and from the remaining 3 subjects with AGE were negative for biofilm formation. Control strains of *E. coli* produced the expected patterns: K12 and EAEC O42 were negative and positive, respectively, for biofilm formation.

3.5. Phylogenetic analysis

MLST conducted on *E. coli* pathotypes from subjects with AGE in Davidson County, Tennessee, revealed that they are genetically related to EPEC and EAEC clinical isolates previously reported in the MLST database (http://mlst.warwick.ac.uk/mlst/). MLST sequences from EPEC and EAEC isolates from children with AGE in Davidson County, Tennessee, were compared to similar sequences from EPEC clinical isolates from subjects with AGE and available in the MLST database (http://mlst.warwick.ac.uk/mlst/). We identified 5 previously reported MLST sequence types and 2 novel sequence types, among DEC isolates (Table 3). The 2 new sequence types ST4599 and ST4600, submitted to the MLST database, corresponded to the EAEC EN1I-0245 and EPEC EN1I-0027 strains (Table 3). The EPEC EN1I-027 MLST also had a new *icd* sequence designated *icd486* that was also submitted to the MLST database. A phylogenetic tree constructed based on cluster W alignments of MLST concatemer sequences from Davidson County was compared with MLST sequences from ancestral *E. coli* strains from groups A, B1, B2, and D. As shown in Fig. 2, most DEC from Davidson County clustered with *E. coli* ancestral group A and B1 strains. The only exceptions were the EPEC EN1I-0034 strain that clustered with ancestral

group B2 and the EPEC EN1I-0027 strain that was not related to any of the *E. coli* ancestral group strains.

EPEC isolates from Davidson County revealed close relationships with previously reported EPEC clinical isolates derived from AGE cases from different worldwide locations. EPEC EN1E-0139 strain with MLST sequence type 342 has a clonal relationship with EPEC Trh37, a Norway strain O177 isolated from a case of diarrhea (Fig. 3). EPEC strains EN1E-0224, EN1E-0241, and EN1I-0032 are also identical and cluster with *E. coli* ancestral group A and specifically with MLST sequence type 10 (ST10), associated with EPEC human pathogens. The remaining EPEC MLSTs we identified are not identical to EPEC from the MLST database, yet they are related (Fig. 3). The EPEC EN1I-0027 strain, for instance, is distantly related to EPEC 109 in the database, a Chinese O15 isolate from a case of diarrhea.

The 4 EAEC clinical isolates clustered with EAEC clinical isolates reported in the MLST database (Fig. 4). Two EAEC study strains formed a clonal group, in addition to identical MLST, and they also share identical O86:H27 serotype. Interestingly, the EAEC EN1E-0191 strain clustered also with ancestral group A, ST10, which is associated with both EPEC and EAEC strains. The EAEC EN1E-0245 strain is closely related to ST10, although it belongs to its own new sequence type ST4599 (Table 3).

4. Discussion

Diarrheagenic *E. coli* are a common cause of infant diarrhea in developing countries, but their impact in pediatric infectious diarrhea in industrialized nations remains unclear (Kotloff et al., 2013). Previous studies in the United States examining the prevalence of DEC have reported several *E. coli* pathotypes including STEC; EPEC; EAEC; and, less frequently, ETEC (Devasia et al., 2006; Denno et al., 2012; Klein et al., 2006; Nataro et al., 2006). Our study identified atypical EPEC and EAEC as the 2 DEC pathotypes represented among patients with AGE in this geographic area. In our cohort, other *E. coli* pathotypes, including STEC strains which can cause HUS complications and mortality (Tarr et al., 2005), were not found.

EPEC has been considered a cause of human disease since the 1940s (Walker-Smith, 1996). The identification of certain genetic determinants,*eae* (encodes intimin, which mediates aggregation) and *bfpA* (encodes the bundle-forming pilus), has aided in understanding EPEC pathogenicity (Jerse et al., 1990). aEPEC is defined as the presence of *eae* without *bfpA* (Trabulsi et al., 2002), and this represented the majority of our samples. While the pathogenic potential of aEPEC strains has been speculative in the past, a recent publication by the Global Enteric Multicenter Study (GEMS) showed aEPEC as the 5th most frequently detected pathogen in patients aged 0–11 months who died of AGE (Kotloff et al., 2013). The 3.8% EPEC prevalence in this study is similar to that reported in other US geographic regions (Denno et al., 2012; Nataro et al., 2006; Caeiro et al., 1999). Additionally, typical EPECs (*eae*+, *bfpA*+) were found most frequently in that age group. Our EPEC-positive specimens were too few to demonstrate any association with age, which has been shown previously (Sakkejha et al., 2013). Interestingly, studies conducted in England and Peru

have shown that aEPEC is often found in children with and without diarrhea, whether in the developed or developing world (Hernandes et al., 2009; Ochoa and Contreras, 2011). Further surveillance studies in the United States that include healthy controls may provide clues on host risk factors as well as EPEC virulence factors that are associated with disease.

Of the 8 positive EPEC specimens, 2 (25%) had O119 serogroups consistent with recognized classic human EPEC serogrups (Levine and Edelman, 1984). One EPEC sample had an O serogroup bovine association (EPEC O35) (Blanco et al., 2005). Three EPEC isolates were nontypeable, and 2 belonged to O serogroups (O6, O108) not previously associated with EPEC strains. This may indicate that emergence of new O serogroups among EPEC strains in humans is associated with disease or with permanent or transient colonization of the human intestine without evidence of disease.

Studies from multiple cities in the United States, as well as cities in Brazil, Peru, and Burkina Faso, have repeatedly demonstrated that EAEC is the most frequently identified DEC found when specimens are tested for the 6 known pathotypes (Klein et al., 2006; Nataro et al., 2006; Bonkoungou et al., 2013; Cohen et al., 2005; Lozer et al., 2013). The 1.9% EAEC prevalence in this study was lower than the one reported in the United States previously (Denno et al., 2012; Nataro et al., 2006). EAEC is typically defined by a “stacked-brick” aggregation pattern on HEp-2 cells directly associated with biofilm formation (Kaper et al., 1997). Increasingly, PCR methods detecting *aggR* (a central regulator of virulence) are being used for identification. Reliable ways of determining whether EAEC causes disease are difficult, as it is also known to cause asymptomatic colonization (Nüesch-Inderbinen et al., 2013). EAEC-positive specimens in this study were observed in younger children, consistent with observations in the United States and developing countries (Kotloff et al., 2013; Klein et al., 2006; Nataro et al., 2006; Bonkoungou et al., 2013; Lozer et al., 2013). The 4 EAEC strains isolated in the current study belonged to 3 serogroups: 2 O86 and 1 each O3 and O92. These serogroups have previously been associated with EAEC diarrheal disease and shown to display aggregative adhesion and biofilm formation (Knutton et al., 1992). Interestingly, only 1 EAEC isolate from 1 subject was positive for biofilm formation compared with EAEC strain control. This may indicate that the remaining EAEC isolates unable to form biofilm may be nonpathogenic intestinal colonizers. Alternatively, they may form biofilm only under host intestinal conditions.

Overall, DEC isolates were susceptible to the antibiotics tested in our study, with the exception of resistance to ampicillin (5/12, 41.6%). Only 1 strain was resistant to at least 3 classes of antimicrobials. In contrast, multidrug resistance was reported among DEC isolates in Iran, Kenia, Mexico, and South Korea (Al Jarousha et al., 2011; Harel et al., 1991; Sang et al., 2012; Unno et al., 2011).

MLST typing of strains reveled associations between *E. coli* clinical isolates from Davidson County, Tennessee, and *E. coli* ancestral groups A and B1 known to be associated with intestinal disease (Gómez-Duarte et al., 2010; Guerra et al., 2014). EPEC clinical isolates demonstrated close MLST relationships with previously reported EPEC strains. Three EPEC and 1 EAEC Davidson County strains belong to MLST ST10, which includes EPEC and

EAEC pathotypes and belongs to the ancestral *E. coli* group A. These phylogenetic findings are strong evidence that EPEC and EAEC isolates are true intestinal pathogens with genetic traits that may favor human intestinal colonization.

The primary limitation of this study is low overall recovery of *E. coli* from diarrheal samples. Specimens were sometimes plated up to 15 days after collection, reducing the efficiency of *E. coli* culture isolation. Our study lacked an analysis of healthy control specimens, so association between *E. coli* pathotypes and disease cannot be definitively ascertained. As new multipanel systems for DEC detection become available, more accurate epidemiological data on DEC in the United States will be reported (Buss et al., 2015).

In summary, we have reported for the first time the presence of EPEC and EAEC *E. coli* pathotypes among children with AGE in Davidson County, Tennessee. The EPEC strains, the most frequently identified, were atypical as they do not carry bundle-forming pilus genes. EAEC strains were positive for both *aggR* and *aaiC* genes, yet only a single EAEC-positive specimen was able to form biofilm. All pathotypes identified were genetically related to EPEC and EAEC pathotypes previously isolated from human cases of AGE. The role of these pathotypes in the epidemiology of AGE in Tennessee and in the United States warrants further systematic investigation. Information about common MLST types, serotypes, reservoirs, and antimicrobial resistance in prevalent EPEC and EAEC will aid in development of strategies to prevent spread and outbreaks.

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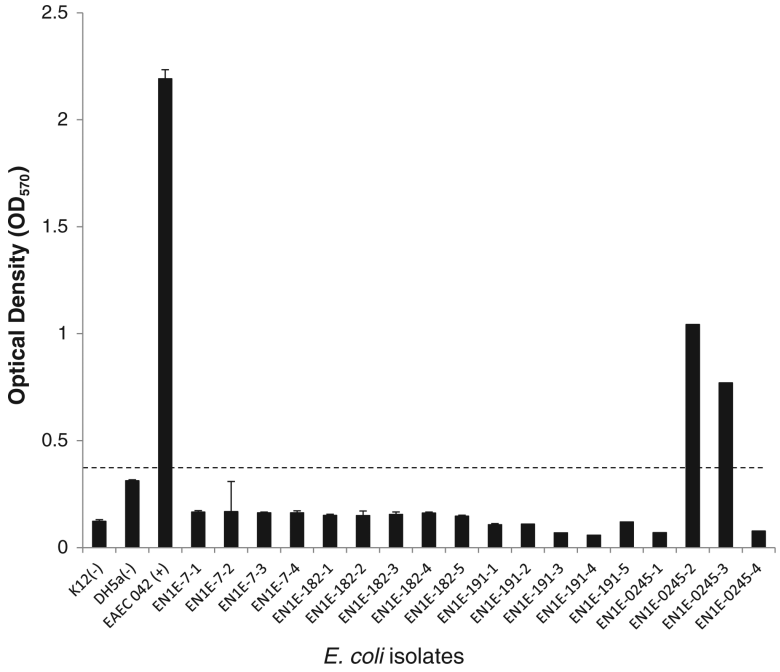

Fig. 1.

Quantitative biofilm formation assay on EAEC isolates. Individual colonies for each case of diarrhea positive for EAEC were tested in the biofilm assay. *E. coli* K12 and DH5 strains are negative controls, and EAEC O42 is positive control for biofilm formation. The optical density is proportional to the amount of bacteria attached to the inert surfaces. Interrupted line is the threshold above which the strain is positive for biofilm formation. The threshold corresponds to the average of the negative controls plus 2 times the SD. In this assay, the threshold was set at an absorbance ($A_{590\ nm}$) of 0.43.

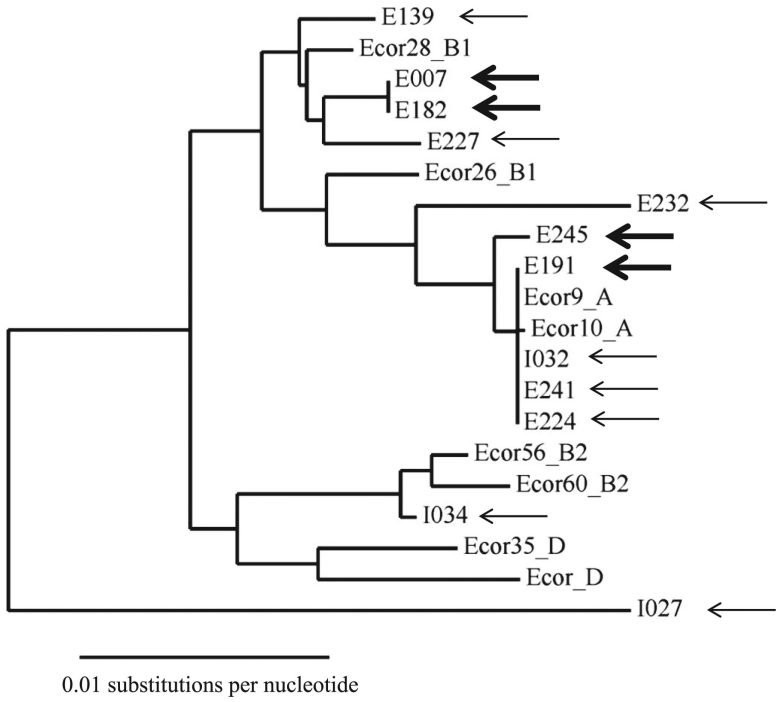

Fig. 2.

MLST phylogenetic tree of *E. coli* intestinal pathotypes isolated from children with AGE. Phylogenetic tree was constructed after assembly and alignment of MLST DNA sequences from diarrheagenic *E. coli* isolates using ClustalW program. *E. coli* MLST sequences from ancestral groups A, B1, B2, and D available at the MLST database (http://mlst.warwick.ac.uk/mlst/) were used as controls. Arrows represent clinical isolates from Davidson County, Tennessee; thin arrows indicate EPEC study strains, and bold arrows indicate EAEC study strains.

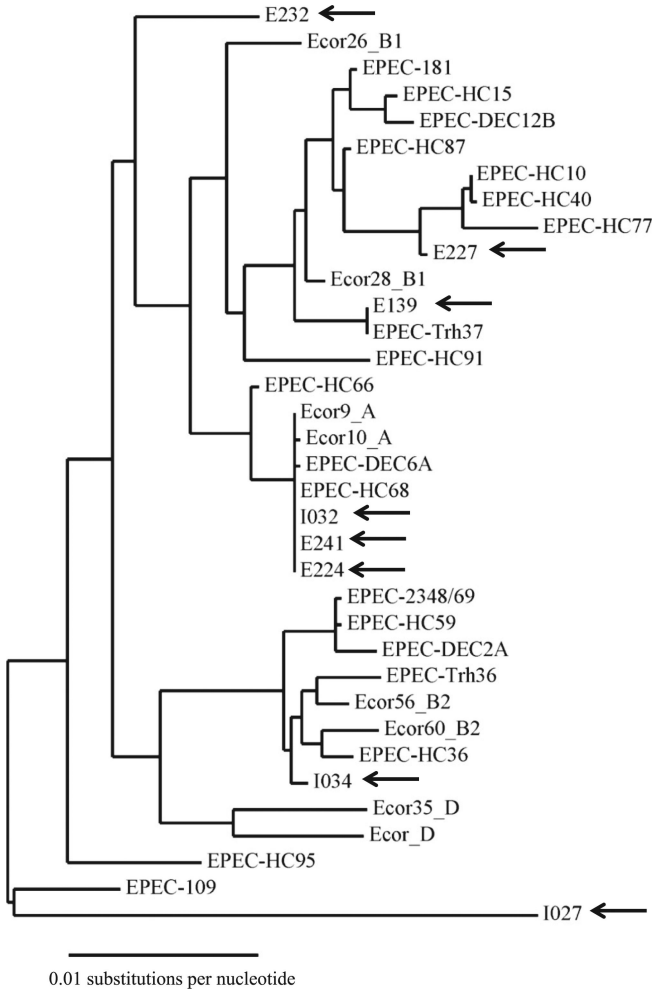

Fig. 3.

MLST phylogenetic tree of EPEC isolates. Phylogenetic tree of the 8 EPEC MLST sequences were compared with MLST sequences from ancestral groups A, B1, B2, and D as well as EPEC sequences, available at the MLST database (http://mlst.warwick.ac.uk/mlst/). EPEC MLST controls included: EPEC HC68; EPEC DEC2A; EPEC 2348/69; EPEC HC10; EPEC HC15; EPEC Trh36; EPEC DEC6A; EPEC DEC12B; EPEC Trh37; EPEC 181; EPEC HC40; EPEC HC36; EPEC HC91; EPEC HC95; EPEC HC59; EPEC HC66; EPEC HC87; EPEC 109; EPEC 219. E. coli ancestral control strain sequences are derived from the ECOR collection and they include clonal group B1, Ecor26 and Ecor28; clonal group A, Ecor9 and Ecor10; clonal group B2, Ecor56 and Ecor60; and clonal group D, Ecor35 and Ecor36. Arrows represent EAEC clinical isolates from Davidson County, Tennessee.

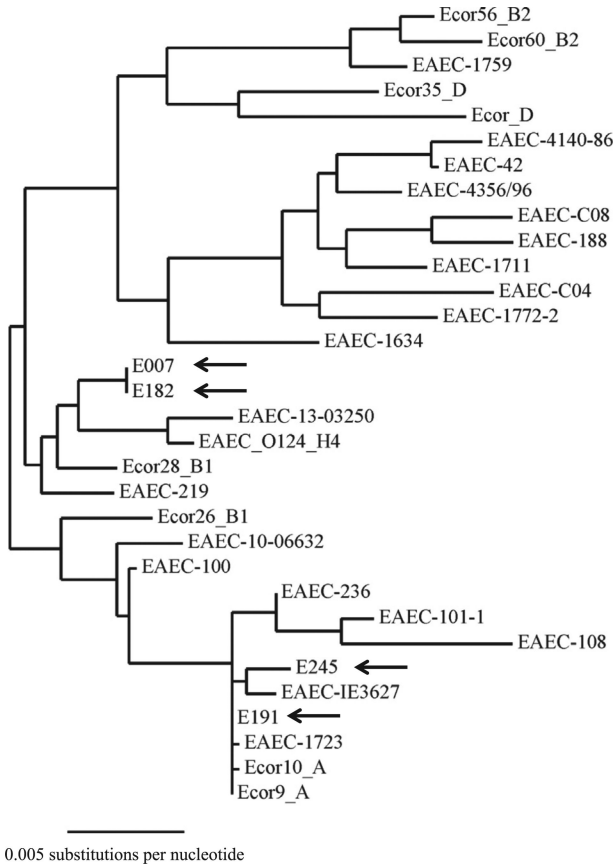

Fig. 4.

MLST phylogenetic tree of EAEC isolates. Phylogenetic tree constructed after assembly and alignment of MLST DNA sequences using the ClustalW program. EAEC MLST sequences from study isolates: EN1E-0007, EN1E-0182, EN1E-0191, and EN1E-0245. *E. coli* MLST sequences from ancestral groups A, B1, B2, and D as well as STEC sequences, available at the MLST database, were used as controls. *E. coli* ancestral control strain sequences are derived from the ECOR collection. EAEC MLST sequences from human cases of diarrhea included: EAEC 4356/96; EAEC 101-1; EAEC 13-03250; EAEC 100; EAEC 236; EAEC C08; 42; EAEC C04; EAEC IE-3627; EAEC 10-06632; EAEC 1634; EAEC 1759; EAEC 1772-2; EAEC 1723; EAEC 108; EAEC 1711; and EAEC 219; 188. Arrows represent EAEC isolates from Davidson County, Tennessee.

Table 1

Demographics of children with acute gastroenteritis.

	Negative for *E. coli*	Positive for *E. coli*	Pathogenic *E. coli*[a]	Total
Sex				
Male	70 (63%)	41 (37%)	9 (8%)	111
Female	57 (60%)	38 (40%)	3 (3%)	95
Age				
<1 year old	46 (65%)	25 (35%)	5 (7%)	71
1 to <2 years old	20 (53%)	18 (47%)	2 (5%)	38
2 to <5 years old	35 (62%)	21 (38%)	3 (5%)	56
5 to <10 years old	24 (67%)	12 (33%)	2 (6%)	36
≥10 years	2 (40%)	3 (60%)	0 (40%)	5
Race				
White	56 (54%)	47 (46%)	10 (8%)	103
Black	61(68%)	29 (32%)	2 (3%)	90
Asian	4 (100%)	0	0	4
Other	6 (67%)	3 (33%)	0	9

[a] Pathogenic *E. coli* are a subgroup of *E. coli* isolates that tested positive by PCR for either EPEC or EAEC.

Table 2

Demographic features of AGE cases positive for diarrheagenic *E. coli*, December 2011–June 2012.

Sample	Subject	Age (months)	Sex	Race[a]	Ethnicity	Month of isolation	*E. coli* pathotype
1	EN1E-0007	18	M	W	NH/NL	December	EAEC
2	EN1E-0139	22	M	W	NH/NL	March	EPEC
3	EN1E-0182	4	F	W	H/L	April	EAEC
4	EN1E-0191	39	M	W	H/L	May	EAEC
5	EN1I-0027	2	M	W	NH/NL	May	EPEC
6	EN1I-0032	27	F	W	H/L	May	EPEC
7	EN1E-0224	41	M	W	NH/NL	June	EPEC
8	EN1E-0227	10	M	B	NH/NL	June	EPEC
9	EN1E-0232	113	F	W	NH/NL	June	EPEC
10	EN1E-0241	65	M	W	H/L	June	EPEC
11	EN1E-0245	8	M	B	NH/NL	June	EAEC
12	EN1I-0034	4	M	W	NH/NL	June	EPEC

[a]Race: W represents white and B represent black. Ethnicity: H/L represents Hispanic or Latino and NH/NL represents non-Hispanic or non-Latino.

Table 3

Genotypic and phenotypic features of diarrheagenic *E. coli* isolates.

Sample	Subject	Pathotype	Virulence genes	Serotype	Sequence type	Antibiotic resistance[b]
1	EN1E-0007	EAEC	*aggR, aaiC*	O86:H27	3570	A
2	EN1E-0139	EPEC	*eae*	O6:H1	342	None
3	EN1E-0182	EAEC	*aggR, aaiC*	O86:H27	3570	A
4	EN1E-0191	EAEC	*aggR, aaiC*	O3:H2	10	A
5	EN1E-0224	EPEC	*eae*	O–:H–	10	A
6	EN1E-0227	EPEC	*eae*	O119:H21	40	A, A/C
7	EN1E-0232	EPEC	*eae*	O108:H9	302	None
8	EN1E-0241	EPEC	*eae*	O–:H–	10	A, A/C
9	EN1E-0245	EAEC	*aggR, aaiC*	O92:H33	ST4599[a]	A, A/C, S
10	EN1I-0027	EPEC	*eae*	O119:H30	ST4600[a]	A
11	EN1I-0032	EPEC	*eae*	O35:H10	10	None
12	EN1I-0034	EPEC	*eae*	O–:H9	779	A, S

[a] New MLST sequence type submitted to http://mlst.warwick.ac.uk/mlst/.

[b] A = ampicillin; A/C = amoxicillin/clavulanic acid; S = sulfamethoxazole-trimethoprim.