Original Article

Enteroaggregative *Escherichia coli* in diarrheic children in Egypt: molecular characterization and antimicrobial susceptibility

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Abstract

Introduction: Little information is available regarding the significance of enteroaggregative *Escherichia coli* (EAEC) in pediatric diarrhea in Egypt.

Methodology: *Escherichia coli* was isolated from stool samples of 62 diarrheic and 43 non-diarrheic (control) Egyptian children. Samples were screened for genes specific for enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), and enteroinvasive *E. coli* (EIEC) using polymerase chain reaction (PCR). Diarrheagenic *E. coli* were grouped phylogenetically using PCR and tested for their susceptibility to antibiotics using the disk diffusion method. Isolates designated as EAEC were examined for eight virulence factors (VFs) using PCR.

Results: EAEC was detected in 19 (30.7%) and 4 (9.3%), EPEC in 2 (3.2%) and 1 (2.3%), and ETEC in 2 (3.2%) and 0 (0.0%) diarrheic and control children, respectively; STEC and EIEC were not detected. Only EAEC was significantly isolated from diarrheic children compared with controls ($p < 0.01$, OR = 4.31).Three or more VFs (multivirulent isolates) were found in 52.6% and 50% of EAEC isolated from diarrheic children and controls, respectively. More than 73% (17/23) of EAEC isolates were identified as belonging to phylogenetic group D. Multiple-antibiotic resistance (resistance to three or more drugs) was observed in more than 91% of EAEC.

Conclusions: Multivirulent EAEC is a significant causative agent of pediatric diarrhea in Egypt, with the majority of isolated EAEC belong to phylogenetic group D. Multiple-antibiotic resistance among EAEC has the potential to be a serious public health problem for the country.

Key words: Enteroaggregative *Escherichia coli*; Virulence genes; Phylogenetic grouping; Antibiotic susceptibility; Diarrhea; Children; Egypt

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Introduction

Diarrheagenic *Escherichia coli* (DEC) are important intestinal pathogens causing a wide variety of gastrointestinal diseases, particularly among children in developing countries. These organisms are differentiated into enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) [1]. Person-to-person transmission and consumption of foods and drinking water contaminated with human waste are the main modes of acquiring DEC infections.

Generally, acute infectious diarrhea is self-limiting and responsive to therapy by oral rehydration. However, antimicrobial therapy is recommended for severely ill patients and for those with risk factors for invasive infection after obtaining appropriate blood and fecal cultures [2]. High frequency of resistance to antimicrobial agents among DEC isolated from children with diarrhea has been reported from several developing countries [3,4]. With the exception of ETEC [5], there little information published on the susceptibility of DEC from Egypt to antibiotics.

The epidemiological significance of different DEC pathotypes in childhood diarrhea varies geographically. Few studies have examined the clinical relevance of ETEC-associated diarrhea in Egypt [5,6]. However, there is little information on the role of EAEC in pediatric diarrhea in the country. The objectives of the present study were to determine the prevalence of DEC pathotypes in diarrheic and non-diarrheic Egyptian children and to determine the susceptibility of the isolated DEC to antimicrobial

agents. In addition, the phylogenetic grouping of the isolated DEC and determination of virulence-encoding genes in EAEC isolates were determined by PCR.

Methodology

Stool and bacterial samples

E. coli was isolated from stool samples obtained from 62 children with acute diarrhea and 43 control children without diarrhea admitted to Ain Shams University and Boulaq hospitals in Cairo, Egypt, during the summer of 2007. Acute diarrhea was defined as three or more loose or liquid stools per day lasting fewer than 14 days. Children were ≤ 5 years of age; > 90% were < 3 years of age. After receiving informed consent from a parent or guardian, a clinical history for each child was obtained. Histories included results of the attending doctor's physical examination and clinical symptoms, including fever, vomiting, abdominal pain, dysentery, convulsions, and dehydration using standard charts. Feeding practices (breastfeeding, artificial supplements, or both [mixed]), history of antibiotic usage, source of drinking water (treated or untreated), and history of travel abroad in the 30 days prior to stool collection were also recorded. Control children were admitted to either hospital with non-gastrointestinal illnesses and had not received antibiotics during the 30 days prior to stool collection. The majority of children sampled were from low-income families. Stool specimens from enrolled children were collected using wide-mouthed sterile plastic containers and transported within two hours of collection to the microbiology laboratory for analysis.

Stool specimens were directly streaked onto MacConkey agar (Difco, Detroit, MI, USA) for isolation of *E. coli*. After overnight incubation at 37°C, three lactose-fermenting colonies and a representative non-lactose fermenting colony with a different morphological appearance were picked, and their identities were determined using a panel of biochemical tests interpreted as previously described [7] and the API 20E system (bioMerieux, Marcy l'Etoile, France).

Genotypic methods

Identification of DEC and screening of virulence factors

Previously reported PCR methods [8-13] were used to screen isolates of *E. coli* for genes associated with DEC. These included: p*CVD432* encoding for EAEC; *eaeA* and *bfpA* for EPEC; *stx1*and *stx2* for Shiga-like toxin 1 (SLT-1) and Shiga-like toxin 2 (SLT-2), respectively, for STEC; *estA1* and *estA2-4* for heat-stable (ST) and *eltB* for heat-labile (LT) toxins of ETEC; and *ipaH* for EIEC. Also, PCR techniques [14-21] were used to screen all *E. coli* isolates for EAEC-associated virulence genes including: *aggR*, a transcriptional activator; *aggA,* fimbriae AAFI; *aafA*, fimbriae AAF II; *agg3A,* fimbriae AAF III; *astA*, aggregative stable toxin 1 (EAST 1); *pet*, plasmid-encoded heat-labile toxin; *aap*, anti-aggregation protein; and *pic*, protein involved in colonization. In addition, ETEC-positive *E. coli* isolates were examined for coli surface antigens (CS) by PCR [13]. Product sizes of PCR amplicons used for distinguishing the different virulence genes associated with DEC are shown in Table 1. Whole-cell lysates, obtained by boiling or using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA), from each isolate were used as DNA templates. Amplification was carried out in a 50 µL reaction mixture containing 3 µM $MgCl_2$,400µM each dNTP, 10 µL 5X Green Go Taq Flexi buffer (Promega, Madison, WI, USA), 2.5 U Go Taq Flexi DNA polymerase (Promega), 1 µL each primer and 5 µL whole-cell lysate. Reactions were performed in a GeneAmp 9700 PCR System (Applied Biosystems Incorporated, Carlsbad, CA, USA).

Phylogenetic grouping of DEC isolates

The assignation of phylogenetic groups among DEC isolates was performed by multiplex PCR of the genes *chuA* and *yjaA* and the DNA fragment TspE4.C2 according to the method described by Clermont *et al.* [22], with minor modifications as follows: denaturation for 5 minutes at 94°C; 30 cycles of 94°C for 30 seconds, 59°C for 30 seconds, and 72°C for 30 seconds; using the same denaturing and annealing steps but increasing the final extension step to 7 minutes at 72°C. The amplification products were separated in 2% agarose gels containing ethidium bromide. After electrophoresis, the gel was photographed under UV light, and the strains were assigned to the phylogenetic groups A (*chuA*-, TspE4.C2-), B1 (*chuA*-, TspE4.C2+), B2 (*chuA*+, *yjaA*+), or D (*chuA*+, *yjaA*-).

Table 1. PCR primer sequences for detection of different virulence-associated genes in *E. coli*

Targetgene	Primerpair (5`- 3`)	PCR conditionfor 30 cycles[a]	Size (bp)	Reference
pCVD432	CTGGCGAAAGACTGTATCAT and CAATGTATAGAAATCCGCTGTT	1 min 94°C, 1 min 55°C, 1 min 72°C	630	8
aggR	CTAATTGTACAATCGATGTA and ATGAAGTAATTCTTGAAT	1 min 94°C, 1 min 42°C, 1 min 72°C	308	14
aggA	TTAGTCTTCTATCTAGGG and AAATTAATTCCGGCATGG	1 min 94°C, 1 min 60°C, 1 min 72°C	450	15
aafA	ATGTATTTTTAGAGGTTGAC and TATTATATTGTCACAAGCTC	1 min 94°C, 1 min 55°C, 1 min 72°C	518	16
agg3A	GTATCATTGCGAGTCTGGTATTCAG and GGGCTGTTATAGAGTAACTTCCAG	1 min 94°C, 1 min 50°C, 1 min 72°C	462	17
aap	CTTTTCTGGCATCTTGGGT and GTAACAACCCCTTTGGAAGT	1 min 94°C, 1 min 52°C, 1 min 72°C	232	18
pic	GGGTATTGTCCGTTCCGAT and ACAACGATACCGTCTCCCG	1 min 94°C, 1 min 52°C, 1 min 72°C	1.175	19
pet	GACCATGACCTATACCGACAGC and CCGATTTCTCAAACTCAAGACC	1 min 94°C, 1 min 56°C, 1 min 72°C	599	20
astA	CCATCAACACAGTATATCCGA and GGTCGCGAGTGACGGCTTTGT	1 min 94°C, 1 min 55°C, 1 min 72°C	111	21
stx 1	CAACACTGGATGATCTCAG and CCCCCTCAACTGCTAATA	1 min 94°C, 1min 55°C, 1 min 72°C	350	9
stx 2	ATCAGTCGTCACTCACTCACTGGT and CTGCTGTCACAGTGACAAA	1 min 94°C, 1min 55°C, 1 min 72°C	110	9
eaeA	AAACAGGTGAAACTGTTGCC and CTCTGCAGATTAACCCTCTGC	1 min 94°C, 1min 52°C, 1 min 72°C	454	10
bfpA	AATGGTGCTTGCGCTTGCTGC and GCCGCTTTATCCAACCTGGTA	1 min 94°C, 1min 60°C, 1 min 72°C	326	11
ipaH	GTTCCTTGACCGCCTTTCCGATACCGTC and GCCGGTCAGCCACCCTCTGAGAGTAC	1 min 94°C, 1min 52°C, 1 min 72°C	619	12
*estA*1 (stp)	ATGAAAAAGCTAATGTTGGCA and TTAATAACATCCAGCACAGGCA	1 min 95°C, 30 s 58°C 1 min 72°C[b]	239	13
*estA*2-4 (sth)	AATTGCTACTATTCATGCTTTCAGGAC and TCT TTT TCA CCT TTC GCT CAG G	1 min 95°C, 30 s 58°C 1 min 72°C[b]	133	13
*eltB*I (lt)	CATAATGAGTACTTCGATAGAGGAAC and GAAACCTGCTAATCTGTAACCATCC	1 min 95°C, 30 s 58°C 1 min 72°C[b]	402	13

[a]For all PCR conditions, a final extension step of 7 min at 72°C was performed.

Table 2. Distribution of diarrheagenic *Escherichia coli* (DEC) from diarrheic children according to age, gender, type of feeding, and source of drinking water

DEC	No. (%) positive								
	Gender		Age (months)		Type of feeding*			Source of drinking water	
	F	M	≤12	> 12	Breast	Artificial	Mixed	Treated	Untreated
Egypt	(n = 26)	(n = 36)	(n = 36)	(n = 26)	(n = 30)	(n = 4)	(n = 2)	(n = 61)	(n = 1)
EAEC	7 (26.9)	12 (33.3)	15 (41.7)[1]	4 (15.4)	12 (40.0)	3 (75.0)	1 (50)	18 (29.5)	1 (100)
EPEC	1 (3.8)	1 (2.8)	1 (2.8)	1 (3.8)	1 (3.3)	0 (0.0)	0 (0.0)	2 (3.3)	0 (0.0)
ETEC	0 (0.0)	2 (5.6)	1 (2.8)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.3)	0 (0.0)
Total DEC	8 (30.7)	15 (41.7)	17 (47.2)	6 (23.0)	13 (43.3)	3 (75.0)	1 (50)	22 (36.1)	1 (100)

*Only children ≤ 2 years of age positive for DEC were included.
[1]EAEC was significantly associated with diarrheic children ≤ 12 months of age compared with diarrheic children > 12 months of age ($p < 0.03$, OR = 3.93).

Table 3. Distribution of virulence genes among diarrheagenic *E. coli* (DEC) isolated from diarrheic and non-diarrheic children (controls)

Virulence (gene, type)*	No. (%) positive among DEC	
	Diarrheic children	Controls
EAEC (p*CVD* +ve)	n = 19	n = 4
aggR	18 (94.7)	4 (100)
aggA	8 (42.1)	3 (75)
aafA	0 (0.0)	0 (0.0)
agg3A	0 (0.0)	0 (0.0)
astA	10 (52.6)	2 (50)
pet	0	0 (0.0)
pic	1 (5.3)	0 (0.0)
aap	10 (52.6)	0 (0.0)
Multivirulent (≥ three genes)	9 (47.4)	2 (50)
EPEC (*eae* +ve)	n = 2	n = 1
Typical(*eae* +veand *bfpA* +ve)	1 (50)	0 (0.0)
Atypical (*eae* +ve and *bfpA* −ve)	1 (50)	1 (100)
ETEC (LT +ve, ST +ve or both)	n = 2	n = 0.0
LT	2 (100)	-
STh	0 (0.0)	-
STp	0 (0.0)	-
CS3	1 (50)	-

**aggR*, a transcriptional activator; *aggA,* fimbriae AAF I; *aafA*, fimbriae AAF II; *agg3A,* fimbriae AAF III; *astA*, aggregative stable toxin 1 (EAST-1); *pet*, plasmid-encoded heat-labile toxin; *aap*, anti-aggregation protein; *pic*, protein involved in colonization; *bfpA*, bundle-forming pilus; LT, heat-labile toxin; ST, heat-stable toxin; CS, coli surface antigens

Table 4. Antimicrobial susceptibility profile of diarrheagenic *Escherichia coli* isolated from children in Egypt with diarrhea or from non-diarrheal controls

Antibiotic	No. (%) resistant			
	EAEC* (n = 23)	EPEC (n = 3)	ETEC (n = 2)	Total (n = 28)
Ampicillin	23 (100)[1]	2 (66.7)	2 (100)	27 (96.4)
Amoxicillin/clavulanic acid	16 (69.6)[2]	0 (00)	1 (50)	17 (60.7)
Imipenem	0 (0.0)	0 (00)	0 (00)	0 (0.0)
Cephalothin	22 (95.7)[3]	1 (33.3)	2 (100)	25 (89.3)
Cefotaxime	18 (78.3)[4]	0 (00)	0 (00)	18 (64.3)
Ceftriaxone	21 (91.3)[5]	0 (00)	0 (00)	21 (75)
Chloramphenicol	17 (73.9)[6]	0 (00)	0 (00)	17 (60.7)
Nalidixic Acid	3 (13)	0 (00)	0 (00)	3 (10.7)
Ciprofloxacin	0 (0.0)	0 (00)	0 (00)	0 (0.0)
Streptomycin	22 (95.7)[7]	1 (33.3)	2 (100)	25 (89.3)
Trimethoprim/sulfamethoxazole	22 (95.7)[8]	0 (00)	2 (100)	24 (85.7)
Tetracycline	18 (78.3)	2 (66.7)	2 (100)	22 (78.6)

*EAEC, enteroaggregative *E. Coli*; EPEC, enteropathogenic *E. Coli*; ETEC, enterotoxigenic *E. coli*
[1]EAEC isolates were significantly more resistant to ampicillin compared with EPEC ($p < 0.005$, OR = undefined).
[2]EAEC isolates were significantly more resistant to amoxicillin-clavulanic acid compared with EPEC ($p < 0.02$, OR = undefined).
[3]EAEC isolates were significantly more resistant to cephalothin compared with EPEC ($p < 0.002$, OR = 44.00).
[4]EAEC isolates were significantly more resistant to cefotaxime compared with EPEC and ETEC ($p < 0.006$, OR = undefined).
[5]EAEC isolates were significantly more resistant to ceftriaxone compared with EPEC and ETEC ($p < 0.0002$, OR = undefined).
[6]EAEC isolates were significantly more resistant to chloramphenicol compared with EPEC and ETEC ($p < 0.02$, OR = undefined).
[7]EAEC isolates were significantly more resistant to streptomycin compared with EPEC ($p < 0.002$, OR = 44.00).
[8]EAEC isolates were significantly more resistant to trimethoprim-sulfamethoxazole compared with EPEC ($p < 0.00002$, OR = undefined).

Antimicrobial susceptibility testing

Isolated DEC isolates were tested for susceptibility against antimicrobial agents using the disk diffusion method [23]. The following antimicrobials were used: ampicillin (10 µm), amoxicillin/clavulanic acid (30 µm), imipenem (10 µm), cephalothin (30 µm), cefotaxime (30 µm), ceftriaxone (30 µm), chloramphenicol (30 µm), nalidixic acid (30 µm), ciprofloxacin (5 µm), streptomycin (300 µm), trimethoprim/sulfamethoxazole (25 µm), and tetracycline (30 µm) (Oxoid, Basingstoke, Hampshire, UK). Screening for extended spectrum beta lactamase (ESBL) production was carried out by the double disk diffusion method with cefotaxime and ceftazidime alone and in combination with clavulanic acid [23].

Statistical analysis

Epi Info version 3.5.1 software (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used to analyze the data.Pvalues were calculated using the χ^2 test; pvalues < 0.05 were considered statistically significant.

Results

DEC were significantly detected among diarrheic children (37.1%, 23/62) compared with control children (11.6%, 5/43) ($p < 0.004$, odds ratio [OR] = 4.48). Of the DEC pathotypes examined, EAEC was found in 19 (30.7%) and 4 (9.3%), EPEC in 2 (3.2%) and 1 (2.3%), and ETEC in 2 (3.2%) and 0 (0.0%) of diarrheic children and controls, respectively; STEC and EIEC were not detected. Only EAEC was significantly isolated from diarrheic children compared with controls ($p < 0.01$, OR = 4.31).

Acute diarrhea lasting for more than one day was recorded for 91.3% of the cases; vomiting and fever (87%), dehydration (82.6%), and abdominal pain (43.5%) were recorded for DEC-positive children with diarrhea. Distribution of DEC isolated from diarrheic children according to age, gender, type of feeding practices, and source of drinking water is shown in Table 2. No significant differences were found in the isolation rates of DEC in relation to gender, type of feeding, or source of drinking water. However, EAEC was significantly associated with diarrheic children one year of age or younger compared with diarrheic children > 12 months of age ($p < 0.03$, OR = 3.93). None of the children included in the present work had traveled abroad in the 30 days prior to stool collection.

Distribution of virulence genes among the isolated DEC

Table 3 shows the distribution of virulence factors in DEC isolated from children with diarrhea and from controls. No significant differences were observed among EAEC from diarrheic children compared with controls for the presence of any of the eight genes examined. More than 95% (22/23) of EAEC from diarrheic and control children carried the *aggR* gene. Three or more VF genes (multivirulent isolates) were found in 47.4% and 50% of EAEC isolated from diarrheic children and controls, respectively. Typical EPEC (*eae*+ve and *bfpA*+ve) were found in 50% (1/2) of EPEC from children with diarrhea. Only genes encoding heat-labile enterotoxins were detected in the recovered ETEC isolates.

Determination of DEC phylogenetic groups

Of the 23 EAEC isolates recovered (including the 4 from controls) a single isolate (4.4%) was identified as belonging to phylogenetic group A, 2 (8.7%) to group B1, 3 (13%) to group B2, and 17 (73.9%) to group D. Of the 3 EPEC isolates (1 from a control) 1 (33.3%) was identified as belonging to group B1 and 2 (66.7%) to group B2. Of the 2 ETEC isolates, 1 (50%) was identified as belonging to group A, and 1 (50%) belonged to group D.

Susceptibility to antimicrobial agents

More than 95% (22/23) of EAEC isolates were resistant to ampicillin, trimethoprim-sulfamethoxazole, and tetracycline. Multidrug resistance (MDR, resistant to three classes of antimicrobial agents or more) was observed in more than 91% (21/23) of EAEC isolates. In addition, EAEC isolates were significantly more resistant to cefotaxime, ceftriaxone, and chloramphenicol ($p < 0.006$, OR = undefined;$p < 0.0002$, OR = undefined; and $p < 0.02$, OR = undefined, respectively) compared with EPEC and ETEC isolates. Furthermore, ESBLs were detected in 78.3% (18/23) of EAEC isolates but in none of the EPEC or ETEC isolates. All EAEC, EPEC, and ETEC isolates recovered in the present study were susceptible to imipenem and ciprofloxacin. The antimicrobial susceptibility profile of DEC isolated from children in Egypt with diarrhea and controls is shown in Table 4.

Discussion

Reports on the prevalence of DEC using molecular methods from North Africa and the Middle East countries are few. Albert *et al.* investigated the

etiology of DEC in hospitalized children with diarrhea in Kuwait [24]. They found no significant difference in the detection of different DEC pathotypes from diarrheic children compared with controls. In the present work, only EPEC, ETEC, and EAEC were detected in stool samples, with only EAEC being significantly found in diarrheic children (30.7%) compared with controls (9.3%) ($p < 0.01$). Compared with our findings, lower prevalence rates of EAEC from diarrheic children have been reported from Iraq (8%), Kuwait (2.6%), Libya (4.1%), and Tunisia (11.3%) [24-27].

Prevalence rates between 1% and 24% for EPEC from diarrheic children using PCR techniques have been reported from several countries in North Africa and the Middle East [24-26]. In the present investigation, EPEC was detected in 3.2% of diarrheic children in Egypt. Previous studies observed that ETEC was a significant cause of severe childhood diarrheal disease in Egypt, with prevalence rates of 10%-20% [6,28]. In this study, only two patients were positive for ETEC (3.2%). It should be noted that previous studies from Egypt used the GM1 enzyme-linked immunosorbent assay for detection of LT and STa [29,30], while we used PCR techniques in the present investigation. Although it is not easy to explain the differences in prevalence rates of DEC pathotypes found in the present and previously mentioned investigations, several factors may contribute to such differences, including geographical locations, populations studied, and quality of sanitation.

In the present study, the three main symptoms (fever, dehydration, and vomiting) were observed in more than 80% of DEC-positive children. The high rates of these clinical symptoms among affected children supports the etiological importance of DEC, particularly EAEC, in Egypt, a region where diarrhea is endemic. A significant association of EAEC with diarrheic children one year of age or younger compared with diarrheic children older than one year of age ($p < 0.03$) was observed as part of this study. Al-Gallas *et al.* in Tunisia reported similar findings [27]. Substantial proportions of DEC were isolated from breastfed children (76.5%) in this study. Although breastfeeding protects from the morbidity and mortality caused by diarrhea in the first few months of life [31], lack of hygiene, sanitation, and other factors may undermine the role of breastfeeding in reducing the prevalence of infectious diarrhea among children, particularly in low socio-economic status communities.

Studies have shown that EAEC possessed different virulence traits and belonged to different phylogenetic groups, indicating their heterogeneity [8,26]. The three predominant genes detected in EAEC from diarrheic children in Egypt were *aggR* (94.7%), *astA* (52.6%), and *aap* (52.6%). Ali *et al.* reported relatively similar findings for *aggR* (90%), *astA* (70%), and *aap* (50%) in EAEC from Libyan children with diarrhea [26].

EAEC isolates with three or more VF genes (multivirulent isolates) were found in 47.4% of EAEC isolated from Egypt (Table 3). A recent study from Libya found similar results, with multivirulence being detected in 50% of EAEC from diarrheic children [26]. Sarantuya *et al*. reported a lower rate (< 30%) of multivirulence among EAEC from children with diarrhea in Mongolia [32].

We found the majority (> 73%) of EAEC (from both diarrheic children and controls) to be associated with phylogenetic group D. Okeke *et al.* reported that EAEC strains (> 80% isolated from diarrheic and control children) from Nigeria were almost evenly distributed among A, B1, and D phylogenetic groups and that only 4.7% of them belonged to group B2 [33]. On the other hand, Pérez *et al.* did not find phylogenetic group D among eight EAEC strains from Costa Rican diarrheic children [34]. The differences in phylogenetic groups among our EAEC isolates and those reported by the above-mentioned studies could be due to different ancestral origins of EAEC in each location.

Knowledge of recent local antibiotic susceptibility patterns of pathogens can assist in choosing the proper treatment when required. We observed high rates of resistance to the commonly used drugs ampicillin, trimethoprim-sulfamethoxazole, and tetracycline among the isolated DEC, which is concerning as treatment may not be suitable with these drugs. High rates of MDR (resistance to three drugs or more) among enteric bacteria are not uncommon in developing countries. The misuse of antimicrobials and their availability over the counter without a prescription is common in developing countries, including Egypt [35,36]. Therefore, it is not unexpected to find high rates (more than 91%) of MDR among our EAEC isolates. Nguyen *et al.* reported similar observations (90% MDR) among DEC (including EAEC) isolated from children [3]. In addition, we found that more than 78% of EAEC and none of the EPEC and ETEC isolates produced ESBL. A study from Kuwait reported a lower rate for ESBL (28.6%) among EAEC from diarrheic children [24].

In conclusion, multivirulent EAEC is a significant causative agent of pediatric diarrhea in Egypt, with the majority of the isolates belonging to phylogenetic group D. Multiple-antibiotic resistance among EAEC is a serious health problem in the country. More studies are needed to determine if EAEC may be prevalent in other cities in Egypt and to address the problem of antimicrobial resistance associated with these organisms.

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