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Dhiarroginic Enteroaggregative, Shiga Toxin-producing *Escherichia coli* and Enterobacteriaceae in Hospitalized Diarrheal Children

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ABSTRACT

Twelve out of 100 stool samples of diarrheal children cases, collected from Abou El- Reesh Hospital, Cairo University, were identified as Enteroaggregative *Escherichia coli* and 7 of them were Shiga toxins producing strains. Samples were divided into five groups (1 to 5 / 20 cases each) according to children ages (<1 to 5 years). The *E. coli* containing samples were examined in association with other food borne bacteria of Enterobacteriaceae. Detection of Enteroaggregative *E. coli* was carried out using the traditional methods and rapid detection of the pathogen, using both kits of HiBio-IDTM, and Hi25TM (Enterobacteriaceae identification kits KB003). Further serological and identification tests were carried out using O and H pool antisera of Escherichia coli Kits. Clump formation as Quantitative biofilm assay and the HEp-2 cell adhesion assay for confirmation of EAEC. Immune Card STAT EHEC Kits was used to detect the (EAEC) shiga toxin (ST) producer strains. Enteroaggregative *E. coli* different serotypes were isolated from group 1 (10 %), group 2 (5%), group 3 (10%), group 4 (15%), and the most infected group was group 5 (20%). Out of the twelve EAEC strains, 4 strains O128:H7 (33%), 3 strains O111:H2 (25%), 2 strains of O127:H2 (16%) and one strain of O126:H7, O126:H6 and O125:H6 (8.3% each) were serologically typed. The results of shiga toxins (ST1 or ST2) production revealed that 5 strains (41%) belong to type ST1 and 2 strains (16%) belongs to type ST2. Dhiarroginic E.coli other than EAEC as EPEC, ETEC and EHEC as strains of pathogenic *E.coli* isolated in this study belonged to about 8 serogroups (O55, O86, O111, O124, O125, O126, O127, and O128).
Enterobacteriaceae as coliform bacteria (100 %), *Salmonella spp* (8.3%), *Citrobacter spp* (8.3%), *Klibsiella spp* (24%), *Proteus spp* (33.3%), *Enterobacter spp* (16.3%), and *Yersinia* enterocolitica (50%), and total aerobic colony count were determined in association with *E. coli* in the positive EAEC stool samples.

Key words: Children, Diarrhea, Enteroaggregative *Escherichia coli*, Shiga toxin and Enterobacteriaceae.

Introduction

Escherichia coli are the predominant facultative organism in the human gastrointestinal tract. Pathogenic forms of *E. coli* can cause a variety of diarrheal diseases in hosts due to the presence of specific colonization factors, virulence factors and pathogenicity associated genes, which are generally not present in other *E. coli*. According to Epidemiologic data and pathogenic characteristics *E. coli* were classified into six pathotypes, Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive. *E. coli* (EIEC), Enteroaggregative. *E. coli* (EAEC), Diffusely adherent *E. coli* (DAEC), and Enterohemorraghic *E. coli* (EHEC), and have been validated as the main diarrheagenic *E. coli* pathogroups (Torres *et al.*, 2005). Enteroaggregative *E. coli* (EAEC) strains are associated with acute or persistent diarrhea among children in tropical and nontropical temperate regions; and have been implicated in food-borne outbreaks, nasocomial infections and travelers' diarrhea, Enteroaggregative *E. coli* heat-stable toxin 1 (EAST1), was first identified in human isolates of EAEC, is a 4.10 kDa peptide. It has been proposed that the mechanism of action of EAST1 is similar to STa in the development of diarrhea, still unclear, and detection of ST (heat labile toxin) and LT (heat stable toxin) genes along with other virulence factors of ETEC strains, as reported by Aranda *et al.* (2004). *Escherichia coli* that produces one or more types of cytotoxins known as Shiga toxin (Stx) or Verocytotoxin (VT) is referred to as Shiga toxin-producing *E. coli* (STEC) or Verocytoxion producing *E. coli* (VTEC) (Nataro and Kaper 1998). STEC is a well-known pathogen as a cause of diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Griffin and Tauxe 1991). Most cases of HC and HUS have been attributed to STEC O157:H7, but the importance of non-O157 STEC is increasingly recognized (Bettelheim, 2007).

Large outbreak of bloody diarrhea complicated by hemolytic uremic syndrome (HUS) in Germany has been confirmed that this epidemic was related to infection by new, unusual Enteroaggregative Shiga toxin/verotoxin-producing *E. coli* 014:H4 strain (Simon *et al.,* 2012). Mojtaba *et al.,* (2014) found the

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occurrence of Enterotoxigenic and Enteroaggregative *Escherichia coli* strains and antibiotic resistance isolates in raw milk (48%) and unpasteurized cheese (48%), in 200 and 50 samples, respectively. In Egypt, Mostafa *et al.* (2014) used polymerase chain reaction (PCR) to detect the genes encoding heat-stable enterotoxin a (STa), heat-stable enterotoxin b (STb), heat labile toxin (LT) and Enteroaggregative heat-stable toxin1 (EAST1) in 120 diarrheic children and found 10% of 120 (10.00%) isolates harbored the gene for EAST1. Abbasi *et al.* (2014) found that 101 out of 715 diarrheal stool samples, in Shiraz hospitals, were due to diarrheagenic *E. coli* and 5 were confirmed as EAEC in patients.

The high prevalence of EAEC isolates in watery diarrhea were identified as serotypes O111:H2, O104:H4 (Bielaszewska *et al.,* 2011), O111:H21 (Dallman *et al.,* 2012), and O127:H4. Enterobacteriaceae other than *E. coli* producing Stx from cases of human disease where stx-phages can infect a range of bacterial hosts wide than expected (Tschape *et al.,* 1995).

Therefore, the aim of this work came up to investigate the incidence of Enteroaggregative and shiga toxin producing *Escherichia coli* in the increasing diarrheal cases within children before 5 years old. Furthermore, the frequency of Enterobacteriaceae associate bacteria was in concern with the EAEC positive samples.

Material and Methods

Collection of samples and grouping:

Sampling (100 clinical diarrheal stool samples) was established at the University of Cairo, Faculty of Medicine, AbouE lReesh hospital during the period May 2012- March 2013.The study enrolment was on 100 patients (n0 = 100) of various ages (grouped 1,2,3,4 and 5, edged <1 to 5 years old/ 20 cases and samples each) and different sexes affected with diarrhea . Diarrheal stool samples were, collected (in sterile plastic cups), transferred to the laboratory and examined as soon as possible (WHO, 1980).

Isolation and identification of E. coli:

Stool samples were directly plated onto eosin methylene blue (EMB) agar (Becton Dickinson, Sparks, MD, USA) and suspected colonies were streaked onto MacConkey agar (Becton Dickinson). After 24 to 48 h of incubation (37°C) putative *E. coli* colonies were picked up and tested by (IMVic) tests for identification and confirmation of *E. coli* (Kumar et al (2008), Edwards and Ewing (1972). The *E. coli* isolates were stored in 5% glycerol supplemented broth at -70°C until use (Iseri, *et al.* (2011).

Rapid Identification of E. coli and Enterobacteriaceae:

Biochemical identification of *E. coli* isolates and Enterobacteriaceae bacteria was carried out using rapid test kits Hibio-ID and Hi25tm (Enterobacteriaceae identification kits KB003, address is HiMEDIA laboratories Pvt. Limited, A-406 Bhaveshwar Plaza, Mumbai-400 086, India**)** in besides of the traditional methods.

Stereotyping of Enteroaggregative E. coli:

The biochemically confirmed *E. coli* strains (isolates) were submitted to the following serological typing:

*Slide agglutination tests using O – antisera***:**

A slide agglutination test was followed using O –antiserum for Escherichia coli Kits (BIORAD, France) polyvalent and trivalent sera against E.coli serogroups. The test is based on agglutination, by specific sera of bacteria possessing the corresponding antigens**.**

H – agglutination of formalin killed culture:

Microtiter plate agglutination of formalin killed culture using H antisera (*E. coli* antiserum H Pool s Kits) was used for serotyping H of the isolated *E. coli* strains according to the supplier instructions (SSI Diagnostica, 2Herredsvejen – Denmark) (3rd Edition, July 2011. 61289).

Biofilm assay:

Bacterial clump formation on liquid cultures was reported as a rapid test to EAEC. This convenient test is based on the biofilm formation of EAEC. To assess biofilm formation for the isolated strains, method, reagents and screening, in 96 well flat bottom microtiter polystyrene plates, were followed according to Albert *et al.* (1993) and Kawano *et al.* (1998).

HEp-2 cell adhesion.

Aggregative adhesion to HEp2-cells was examined as described by Jenkins *et al.* (2006).

Shiga Toxins Production test

E. coli strains were tested for shiga toxin production using Immuno Card STAT EHEC Kit (Meridian Bioscience, Inc. USA). This is a rapid test for detecting shiga toxin production by *E.coli*, not only for shiga toxin but also to differentiate between the two types of shiga toxin (shiga toxin type 1, ST1 and shiga toxin type 2 ,ST2) in broth methods, according to the kit supplier instructions.

EAEC in association with Enterobacteriaceae and other bacteriological criteria:

The presence of EAEC in the diarrheal stool samples was studied in association with other bacteriological criteria such as: total aerobic colony count (TACC), and Gram-negative bacteria, Enterobacteriaceae, such as coliform bacteria, *Yersinia enterocolitica, Salmonella* spp., *Citrobacter spp*, *Klibsiella spp*, *Proteus spp*, and *Enterobacter* spp as follow:

Total aerobic colony count (TACC):

Total aerobic colony count (TACC) was carried out as the conventional method (FDA, 2002) using plate count agar (Oxoid).

Determination of coliform bacteria and E. coli:

Coliform group was determined using solid medium method plates of violet red bile agar (VRBA) according to the method reported by FDA (2002). Suspected colonies were transferred (loopful) into tubes of MacConkey broth medium (Oxoid, England.). Positive acid and gas tubes were further transferred into EC broth. Positive tubes were streaked onto MacConkey agar (Merck, Germany) according to APHA (1976) and tested for IMViC test for typical *E.coli* and other coliform bacteria.

Isolation and identification of Salmonellae:

Stool samples (25g or ml) were transferred into 225 ml (or 1/10 portion) of lactose broth, which in turn transferred after incubation to 10ml of selenite cystein broth (SC) (Oxoid) whic incubated at 37 ºC for 72h. Plates of *Salmonella & Shigella* ager (SS agar) (Oxoid) were streaked every day (for three days) and incubated at 37º C for 24h, (FDA, 2002). Suspected colonies of *Salmonella spp* were biochemically and serologically identified according to FDA (2002) and APHA (1976).

Detection of Yersinia enterocolitica:

Stool samples (25g or ml) were transferred into 225 ml (or 1/10 portion) of peptone sorbitol bile broth (PSBB)(M120) (Oxoid). After incubation 0.l ml of PSBB was spread onto the surface of Yersinia selective agar medium (Oxoid code CM653) supplemented with Yersinia selective supplement (SR 109), FDA (2002).

Isolation and identification of other Enterobacteriaceae bacteria:

Isolation and identification of *Citrobacter spp*, *Klibsiella spp*, *Proteus spp*, and *Enterobacter* spp. were carried out according to the methods and media outlined by FDA (2002).

Statistical Analysis:

Statistical analyses were performed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability to $P \leq 0.5$ was used establish the statistical significance.

Results and Discussion

Incidence and distribution frequency of Enteroaggregative E. coli in different ages of diarrheal children stool samples.

Results in Table (1) revealed that 12 out of 100 diarrheal children cases (stool samples) were found to contain Enteroaggregative *E. coli* (EAEC), in percentage of 12%. Also, the results illustrate the instance of Enteroaggregative *E. coli* (EAEC), which was isolated from the 5 groups (20 cases/ each) in percentages of 10% for group 1 (Under one year's age), 5% for group 2 (one - two year's age), 10% for group 3(two – three year's age) 15% for group 4 (three - four year's age) and 20% for, the most infected group5 (four – five year's age).
The incidence of Enteroaggregative *E. coli* (EAEC) (12%) isolated in the current study from different children aged groups (<1-5 years) was lesser than that obtained by Ali *et al.* (2014) who found EAEC in diarrheic children (30.7%). Meanwhile, the lower the prevalence rates of EAEC from diarrheic children have been reported in Iraq (8%), Kuwait (2.6%), Libya (4.1%), and Tunisia (11.3%) and many other countries, (Albert *et al.,* 2009 and Kawano *et al.,* 1998). Several factors may contribute to such differences, including geographical locations, populations knowledge and, hygiene quality and sanitation. Therefore, lack of hygiene, sanitation, and

other related factors may determine the role of breastfeeding in reducing the EAEC prevalence of infectious diarrhea among children, particularly in low socio-economic status communities.

Concerning children ages, the most EAEC infected ages were in group 5 (4 – 5 year's,) 4/20 samples (20%),while the lowest infected ages were in group 2 (1 - 2 year's), 1/20 samples (5%) . In this respect, findings agree with Ali *et al* (2014) for DEC incidence in diarrheic children according to age, and gender. Moreover, EAEC in the current study reveal significant difference in ages associated with diarrheic children <1 year age (16.7%) compared with diarrheic children > 12 months of age (83.3%). Al-Gallas *et al* (2007) in Tunisia reported similar findings that emphasize the need of more hygiene control in developing countries for children feeding in different ages.

Table 1: Incidence of Enteroaggregative *E. coli* in 5 aged groups of diarrheal children stool samples.

Type of Samples	Number of samples analyzed	Incidence percentage of EAEC	
		Number	%
Group 1 (Under one year's age)	20 samples	2	10
Group 2 (From one - two year's age)	20 samples	1	5
Group 3(From two – three year's age)	20 samples	2	10
Group 4 (From three - four year's age)	20 samples	3	15
Group 5 (From four – five year's age)	20 samples	4	20
Total samples	100	12	12 %

Serological Identification of Enteroaggregative E. coli strains isolated from diarrheal children stool samples:

Results in Table (2) show the serological identification of 12 Enteroaggregative *E. coli* (EAEC) isolates from 100 diarrheal stool samples. They (12 strains) were serologically identified as 2 strains of EAEC in Group 1: as one strain O111: H2 and one strain O128:H7 in percentage of (16.6 %). Only one strain of EAEC from Group 2 was identified as O126:H7 (8.3 %). Also, 2 strains of EAEC from Group 3 were identified as O127:H2 (16.6 %). Further, 3 strains of EAEC isolated from Group 4 were identified as O128:H7, O125:H6 and O126:H6 (25 %). Meanwhile, the most infected group was Group 5 where 2 strains were identified as O111:H2 and two strains as O128:H7, in a percentage of 33.3 %.

Therefore, as shown in Table (2) results revealed that EAEC O128:H7 (33.3%) strain plays an important role in infection of children followed by EAEC O111:H2 (25%). However, the obtained results indicated that the incidence of EAEC in the current study was lower than that founded by Mohamed *et al* (2011). They indentified serogroups from *E. coli* isolated from 77.27% (34 of 44) of children with diarrhea, compared with 6.25% (8 of 128) of those without diarrhea (P = 0.001). These strains of pathogenic *E.coli* belonged to 12 serogroups (O26, O55, O86, O111, O114, O119, O124, O125, O126, O127, O128, and O142). Meanwhile, they reported that children with diarrhea, O111 serogroups E.coli (27.27%) was the most prevalent, followed by O142 (20.45%) and O127 (6.81 %). Also, the incidence of O55, O111, O126, O127 and O142 serogroups EPEC in diarrheal stool samples were highly significant. However, our findings differ from other previous studies in Bangladesh (Albert *et al.,* 1995), Saudi Arabia (El-Sheikh and El-Assouli, 2001) and Uruguay (Torres *et al*., 2001). Sharmin Zaman et al (2015) studied, water for irrigation and washing/rinse for presence of *E. coli* O157:H7 and Salmonella spp and found that, contamination with *E. coli O157H7*, therefore; there is a risk of contamination of final products. Foodborne outbreaks involving green vegetables contaminated by water have been reported in several studies around the world so its conceder the main source of children and adult infection.

Table 2: Serology identification of Enteroaggregative *E. coli* strains isolated from 5 aged groups of diarrheal children stool samples.

Group Age	Strain serotype	Strain No.	Incidence and percentages of EAEC	
			N0. and % of total samples, n100	% of EAEC (12)
Group 1 (Under one year's age)	O111:H2 H128:H7	1 1	2	16.6
Group 2 (From one - two year's age)	O126:H7	1	1	8.3
Group 3 (From two – three year's age)	O127:H2	2	2	16.6
Group 4 (From three - four year's age)	O128:H7 O125:H6 O126:H6	1 1 1	3	25%
Group 5 (From four – five years age)	O111:H2 O128:H7	2 2	4	33.3%
Total samples			12	100

In many countries, diarrhea caused by bacterial pathogens especially *E. coli* remains one of the major causes of morbidity and mortality in infants and young children (Al-Braiken, 2008). Although in developed and a few developing countries, recent improvements in biological techniques have drastically increased the rate of diagnosis, isolation of bacterial pathogens and consequently reduced the global death rate due to bacterial diarrhea diseases (Sarantuya *et al*., 2004).

Shiga toxins producing strains of Enteroaggregative E. coli in diarrheal children stool samples.

Results as shown in Table (3 and 4) revealed that not all of the serotyped (O-H) Enteroaggregative *E. coli* strains were shiga toxin (ST) producing strains. Since, 41.7% of these strains could produce shiga toxins (types ST1 or ST2), while 7 out of 12 strains (58.3%) could not. Also, Tables (3 and 4) showed that ST1 was incidentally produced by EAEC O111:H2 2 strains in groups (1 and 5), 16.6%, EAEC O127:H2 in group (3), 8.3%, and EAEC O128:H7 in groups (4 and 5), 16.6%, respectively. However, strain EAEC O128:H7 played an important role in shiga toxins production not only for the incidence in two groups (4 and 5) of the children , more than 3 years old, but also in producing the two types ST1 and ST2 by the same strain,16.6%, that might strengthen the morbidity or mortality due to the infection. Furthermore, results as shown in Table (4) reveal that 41.7 of the EAEC strains produced ST1, while 40% of these strains produced ST2, exclusively.

Hence, EAEC O128:H7 strain was harmful strain which produce different types of shiga toxins, ST1 and ST2, and incident in two groups of diarrhea children. EAEC O111:H2 strain produce shiga toxin from one type, ST1 only. Similar studies found that total of 76 *E. coli* strains isolated from human with diarrhea harboring the virulence genes including shiga toxins ST1 and ST2 and the Enteroaggregative *E. coli* was found in most human strains in Enterotoxigenic strains (O6:H16), Enteroaggregative strains (O126:H27) and shiga toxins Enterohemorraghic strains (O157:H7) and (O26:H11), (Shabana et al. 2014) and O111:H2 (Tim Dallman *et al.,* 2012). However, the Enteroaggregative Shiga toxin-producing *Escherichia coli* of serotype O104:H4 and O157:H7 , as new harmful strains, were not detected in the current study, while they were detected in Germany, Belgium and Luxembourg and other different countries causing several outbreaks and sporadic cases (Rauw *et al,* 2014 and Piérard *et al*, 2012).

Table 3: Incidence of EAEC serotypes and shiga toxin producing strains in 5 edged diarrheal children stool samples.

Type of positive Samples	Shiga toxin producing Strain Type	Type of shiga toxin		Incidence percentage	
		shiga toxin 1	shiga toxin 2	No. and % Of 100 stool samples	% of 12 EAEC* samples
Group 1 (Under one year's age)	O111:H2	+	-	1	8.3
Group 2 (From one - two year's age)	O126:H7	-	-	0	0
Group 3 (From two – three year's age)	O127:H2	+	-	1	8.3
Group 4 (From three-four year's age)	O128:H7	+	+	1	8.3
Group 5 (From four – five years age)	O111:H2 O128:H7	+ +	- +	2	16.6
total EAEC samples	12	5	2	5	41.7

** 7 out of 12 EAEC strains , 58.3%, were not shiga toxins production*

Table 4: Incidence of EAEC serotypes and shiga toxins producing stains in the total and *E. coli* containing stool samples of diarrheal children

EAEC serotypes	Incidence of serotypes			Incidence of shiga toxins (ST)					
				ST1			ST2		
EAEC	No	% Total (100)	% EAEC (12)	No	% Total (100)	% EAEC (12)	No	% Total (100)	% EAEC (12)
O128:H7*	4	4	33.3	2	2	16.6*	2	2	16.6*
O111:H2	3	3	25	2	2	16.6	-	0	0
O127:H2	2	2	16.6	1	1	8.3	-	0	0
O126:H7	1	1	8.3	-	0	0	-	0	0
O126:H6	1	1	8.3	-	0	0	-	0	0
O125:H6	1	1	8.3	-	0	0	-	0	0
Total	12	12	100	5	5	41.7	2	2	16.6

** 2 out of 4,EAEC O128:H7 strains were producing ST1 & ST2 by the same strains = 40% of 5 EAEC shiga toxins producing strains*

EAEC other Dhiarroginic E. coli strains from diarrheal children stool samples:

Table (5) showed that Group 1 of diarrhea children (Under one year's age) is the lowest children patient age infected with dhiarroginic *E. coli* group between the five groups of children patient age, in vice versa in Group 5 (From four – five years age) are the most children patient age infected with dhiarroginic *E. coli* group between the five groups of children patient age. Group 1 infected with 2 isolates of dhiarroginic *E. coli* groups, EHEC O55H:7 and EPEC O126:H11. Also results reveal that EPEC are the most incidence strains between dhiarrogenic *E. coli* and O128:H6 strain was the most incidence strain between EPEC, since it was isolated from group 2, group 4 and group 5. On the other hand, EHEC are the seconded incidence between dhiarrogenic *E. coli* and O126:H11 strain is the most incidence strain between EHEC, isolated from group two, group four and group fiveand the most hazardous strain O157:H7 was found only in group 3. While ETEC group was fewer incidences than EHEC and EPEC, and *E.coli* O128:7 strain was the most incidence strain between ETEC strains. EIEC was the lowest incidence between the dhiarrogenic *E. coli* isolated from diarrheal children stool samples.

Acute diarrheal disease is an important health problem among children. Enterotoxigenic *Escherichia coli* are a common cause of childhood diarrhoea in the developing world where sanitation and clean supplies of drinking water are inadequate. Dhiarroginic *E. coli* groups causes a divers of symptoms as watery diarrhoea, which can range from mild, self-limiting disease to severe-purging disease, generating severe dehydration that could lead to hospitalization and death (Gonzales *et al.,* 2013; Vilchez *et al.,* 2014).

Mohamed *et al.* (2011) who studied 125 bacterial isolates obtained from diarrheagenic stool samples, 87 bacterial isolates (69.6%) were identified as *E. coli*. Also, , Bodhidatta *et al*. 2010) reported that diarrheagenic *E. coli* was the major enteropathogenic bacteria in group aged (3-5) years and commonly isolated from cases in age under five years. Iman *et al.* (2011) Study Enteropathogenic *Escherichia coli* (EPEC) causing diarrhea in children in Cairo, Egypt, Children younger than five years with diarrhea. Out of 134 patients 5.2% of them revealed EPEC in the faecal samples, as confined with the obtained results. However, our EPEC group frequency showed some variations compared with results of other studies, Bodhidatta *et al*. (2010). Similarly, serotypes O55:H12, O86:H48, O127:H21, O142:H48, O126:H48, and O126:H19 were significantly associated with diarrhea in children (Kandakai-Olukemi 2009). Also, Serotypes O55:H6 and O111:H2 were reported as most frequent isolates in different geographical areas Botelho *et al.* (2003) and Elias *et al.,* 2002).

Our study agree with Mohamed *et al.* (2011) Indicated that serogroups from *E. coli* isolated from 77.27% (34 of 44) of children with diarrhoea, compared with 6.25% (8 of 128) of those without diarrhoea .The strains of pathogenic *E.coli* isolated in this study belonged to about 8 serogroups (O55, O86, O111, O124, O125, O126, O127,and O128) were also similar to the previous study, 12 serogroups, who reported the incidence of O55, O111, O126, O127 and O142 serogroups of EPEC in diarrhoeal stool samples were highly significant. Although diarrheagenic *E. coli* pathotypes are of public health relevance, they are not routinely sought as enteric pathogens in clinical laboratories worldwide; thus, their incidence in children less than 2 years of age and their importance in community-acquired diarrhea are generally unknown, particularly in areas of endemicity Estrada-Garcia *et al.* (2009).

Finally, the use of antibiotics in general is of minor importance and has been criticized on the grounds of drug toxicity and the risk of increased wide spread antimicrobial resistance Cheesbrough *et al*. (2004).

Table 5: Incidence and frequency distribution of other Dhiarroginic E. coli strain from diarrheal children stool samples from Abou El-Reesh Hospital

Type of positive Samples	EHEC		EPEC		EIEC		ETEC	
	No of Strain	Strain type	No of Strain	Strain type	No of Strain	Strain type	No of Strain	Strain type
G1 (Under one year's age)	1	O55:H7	1	O26:H11	0	-	0	-
G2(From one - two year's age)	1	O126:H11	1	O128:H6	1	O124:H_	1	O128:H7
G3 (From two – three year's age)	1	O86:H11 O157:H7	1	0127:H7	0	-	1	O127:H2
G4 (From three - four year's age)	1	O126:H11	2	O128:H6 O126:H_	1	O124:H30	1	O128:H7
G5(From four – five years age)	1	O126:H11	3	O128:H6 O125:H21	1	O124:H30	1	O128:H7
Total samples	5		8		3		4	

Enteroaggregative E. coli in diarrheal children stool samples in association with, other pathogenic bacteria, Enterobacteriaceae:

Results in Table (6) showed the association and incidence of Enteroaggregative *E. coli* and other related bacteria, as Enterobacteriaceae, in the same positive (EAEC) diarrheal children stool samples. Statistical analysis revealed that means with the same letters are not significantly different ($p \leq 0.05$), whereas values are means± standard error. Incidence of EAEC, as previously reported, out of one hundred (100) diarrheal stool samples, 12 (12%) positive for EAEC had further bacteriological analysis determine the role and substantial association between these bacteria. The common indicator total aerobic colony count, TACC, of the diarrheal samples ranged from 7.30 to 6.11 log cfu/g. However, infection in group 5 of the children showed that this group was the worth as it was not only the most EAEC contaminated group (33.3%) but also mostly infected by the most hazardous pathogenic microorganism, *Salmonella spp* (8.3%, 45s/100), and other relate bacteria as coliform (100%), *Y. enterocolitica* (16.6%), *Proteus spp* (16.6%) and *Citrobacter spp.*(8.3%). Meanwhile, the safer groups due to EAEC infections and incidence of other Enterobacteriaceae bacteria, were group 2 (8.3%) then group 3 (16.6%) and followed by group 4 (25%). Also, group 1 of the children EAEC infected (16.6%) was the lowest incidence of infection related bacteria as Enterobacteriaceae.

Generally, EAEC infected children with diarrhea showed an association between the incidence EAEC and the coliform (100%), *Y. enterocolitica* (50%), *Proteus spp* (33.3%), *Enterobacter spp* (25%), *Klibsiella spp* (24%), *Citrobacter spp.* (8.3%) and the most hazardous and common diarrheal bacteria *Salmonella spp* (8.3%).

Thus, this investigation revealed the highest incidence and association occurred by *Y. enterocolitica*, *Proteus spp, Klibsiella* spp and *Enterobacter* spp. While the lowest incidence was occured by *Salmonella spp and Citrobacter spp* .Similar study was carried out by Mohamed *et al.* (2011) who found that. *Escherichia coli* was the most commonly-isolated organism from diarrhea and non-diarrhea stools (88%) and (85.3%), respectively. The most Enterobacteriaceae associated *E. coli* bacteria were *Klebsiella* spp. and *Shigella* spp. (28 %), (18%) and (22%), (14.6%), respectively, whereas, *Proteus* spp. was lesser commonly isolated from diarrhea and non diarrhea samples. Sharmin Zaman *et al.* (2015) studied thirteen categories of herbs and tea which are from the main source of food for adult and children and analyzed for total aerobic population (TAB), total coliform population and presence of *E. coli*, *E. coli O157:H7* and *Salmonella* spp. The results of the distribution of natural aerobic population, coliform population and presence of *E. coli*, *E. coli* O157:H7 and *Salmonella* spp in different fresh and dry herbs; water and manure soil. Higher aerobic bacterial count was recorded as 6.9 log CFU/g. coliforms, *E. coli*, and *salmonella* was observed throughout the studyThe bacterial pathogens usually in association with *Escherichia coli,* in diarrhea infections include *Shigella, Salmonella, Campylobacter, Yersinia, Aeromonas* (Seung Hak *et al.,* 2006). Thus in the developed countries, the mortality rates have declined considerably in recent times due to improvement in general hygiene and advances in health care for infants and young children (Presterl *et al*., 2003).

Table 6: Enteroaggregative E. coli in association with other pathogenic microorganisms, Enterobacteriaceae, in Diarrheal stool samples**.**

Sample Type	No. of positive samples / Total samples	Food borne micro organism from Enterobacteriaceae							Total aerobic colony count (TACC) Log cfu/ml or gm	Enterobacteriaceae Log cfu/g
		Proteus spp Log cfu/ gm	*Klebsella spp* Log cfu/ gm	*Enterobacter spp* Log cfu/ gm	Coliform Log cfu/ gm	*Citrobacter* Log cfu/ gm	*Salmone -lla* spp	*Y. enterocolitica*		
Group 1	32S /100	0.0c	0.0c	0.0b	4.3a± 0.33	0.0a	ND	0.0b	5.7 c ± 0.33	0.0 d
Group 1	47S /100	0.0c	0.0c	0.0b	3.5a± 0.21	0.0a	ND	0.0b	6.47a ± 0.06	0.0 d
Group 2	50S /100	2.30 a± 0.17	0.0c	0.0b	4.5c±.25	0.0a	ND	2.8a±0.12	6.39 a ± 0.35	2.00 b ± 0.0
Group 3	43S /100	2.00 b± 0.0	2.19 b ± 0.057	0.0b	4.2c±0.31	0.0a	ND	2.4c±0.18	7.17 b± 0.017	0.0d
Group 3	61S /100	0.0c	2.24 b± 0.23	0.0b	4.2c± 0.18b	0.0a	ND	2.7a±0.11	7.8 b ± 0.09	0.0d
Group 4	27S /100	0.0c	0.0c	0.0b	4.5a±0.30	0.0a	ND	0.0b	7.30 b ± 0.17	(+)0.0d
Group 4	25S /100	0.0c	0.0c	0.0b	4.1a±0.20b	0.0a	ND	2.8a±0.16	6.17 a± 0.066	1.00 c ± 0.0
Group 4	68s /100	0.0c	0.0c	2.89a±0.17	4.30a±0.33	0.0a	ND	0.0b	5.69 c ± 0.35	0.0d
Group 5	29 S/100	0.2.49c	0.0c	2.6 a ±0.15	4.3b±0.2	0.0a	ND	2.39c±0.19	5.91 c ± 0.52	0.0 d
Group 5	45 S /100	0.0c	0.0c	0.0b	4.2b±0.35	2.92b±0.35	+	0.0b	6.11a ± 0.09	2.60 a ± 0.55
Group 5	67 S/100	2.10 b± 0.0	0.0c	0.0b	4.35b±0.21	0.0a	ND	0.0b	7.17b ± 0.024	0.0 d
Group 5	70 S/100	0.0c	0.0c	0.0b	4.6c± 0.18	0.0a	ND	2.1c±0.15	7. 7b ± 0.066	0.0 d
No. of total samples	12/100	4/12	2/12	2/12	12/12	1/12	1/12	6/12	12	3/12
(%). of positive samples	12	33.33	24.0	16.66	100.0	8.33	8.33	50.0	12	25

ND (Not detected), (+) positive, stool and group 1(Under one yrs age), group 2(from one- two yrs), group 3(from two-three yrs), group 4(from three-four yrs), group 5(from four-five yrs).

Conclusions

This study revealed that Enteroaggregative *E. coli* are one of the major causes of acute diarrhea in children in Egypt, especially at the younger ages. We recommend extending the study to target more virulence genes in a large group of underline children with diarrhea. This might greatly facilitate the process to develop vaccines against Enteroaggregative *E. coli* which should be considered a public health issue in Egypt as well as other developing countries.

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