

*Research Article***Diarrhea genic Escherichia Coli in children with acute gastroenteritis at Minia Governorate.****Rasha M. Mahmud Khairy, Soha S. Abd El-Raheem and Zahra A. Fathy.**

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Abstract

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. The aim of the current study was to isolate *E. coli* strains from stool specimens of gastroenteritis kids. The study includes children below 5 years old, whose main complaint was acute diarrhea, characterized by the occurrence of three or more, loose, liquid, or watery stools in a 24h period in addition to one or more symptoms of diarrhea, (nausea, vomiting, abdominal cramps or fecal urgency). This study was carried out in the Microbiology and Immunology department, Faculty of Medicine, Minia University, in the period from May 2017 to December 2018. Stool samples were collected in sterile bottles and the stool characters (loose, watery, formed, a mucoid appearance, or bloody) were reported. Stool specimens were directly streaked onto Mac-Conkey agar (Difco, Detroit, Mich.) for isolation of *E. coli*. The identities of these isolates as *E. coli* were confirmed by different biochemical tests. *E. coli* isolates only were included. Antimicrobial susceptibility to the most common antibiotics was performed in the study isolates.

Key words: nausea, vomiting, abdominal cramps or fecal urgency**Introduction**

Diarrheal diseases are one of the leading causes of morbidity and mortality all over the world and account for more deaths in early childhood than any other etiology (Global Burden of Disease Study 2013; Alexander and Blackburn, 2013). Diarrhea is defined as the passage of three or more loose or watery stools per day (or more frequent passage of stool than is normal for the individual). Gastroenteritis in Children caused by Viruses as (Rotaviruses, Noroviruses (Norwalk-like viruses), by Bacteria as (Campylobacter jejuni, nontyphoid Salmonella spp, enteropathogenic Escherichia coli (*E.coli*), Shigella Yersinia enterocolitica, shiga toxin producing *E coli*, and also *Vibrio cholera*) and by Protozoa and Helminths (Elliott, 2007). Determination of diarrhea etiology and antibiotic susceptibility patterns of diarrheal pathogens and improved hygiene are important for clinical management and controlled strategic planning to reduce the burden of infection (Elliott, 2007). children are self-limiting and do not require the use of medications (Kleinman et al., 2009). The decision to treat a child with acute gastroenteritis and the choice of antimicrobial drug is challenging. Antibiotic selection is based on two major considerations: the

chance of obtaining microbiological results, including resistance pattern, and the severity of clinical conditions. The WHO recommends treating all episodes of blood in the stools with antibiotics and to use ciprofloxacin as the first-line drug. Alternatives are azithromycin, and ceftriaxone (WHO,2005). Fluoroquinolones are often empirically used in adults, and cephalosporins are used to treat children with suspected bacterial acute gastroenteritis. For parenteral therapy of diarrhea, ceftriaxone or ciprofloxacin may be considered, as both are effective against Gram-negative bacteria (Giannattasio et al., 2016). Antimicrobial resistance results in reduced efficacy of antibacterial, making the treatment of patients costly and difficult, or even impossible (Tzouveleki et al., 2014).

Methods

The study includes children below 5 years old, whose main complaint was acute diarrhea, characterized by the occurrence of three or more, loose, liquid, or watery stools in a 24 h period in addition to one or more symptoms of diarrhea, (nausea, vomiting, abdominal cramps or fecal urgency). This study was carried out in the Microbiology and Immunology department,

Faculty of Medicine, Minia University, in the period from May 2017 to December 2018. Stool samples were collected in sterile bottles and the stool characters (loose, watery, formed, a mucoid appearance, or bloody) were reported.

Bacterial isolates:

Stool specimens were directly streaked onto Mac-Conkey agar (Difco, Detroit, Mich.) for isolation of *E. coli*, EMB agar and chromogenic agar, 100 samples yielded significant bacterial growth with *E. coli* strains. The identities of these isolates as *E. coli* were confirmed by different biochemical tests include.

Indole, Methyl red test, Voges-Proskauer, Citrate utilization test, Sugar fermentation test, Urease test: For long term storage, bacterial isolates were incubated in nutrient broth at 37 °C for 24 hours, and then 1 ml of cultured broth was added to 1 ml of 70% sterile glycerol at -20 °C until being used again.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility of the isolates was determined by disk diffusion method according to CLSI guidelines" (CLSI, 2015): The used antimicrobial discs were; ceftriaxone (CRO) 30µg, amoxicillin/clavulanic acid (AMC) 30µg, ceftazidime (CAZ) 30µg, Meropenem 10µg (MEM) 10µg, amikacin (AK) 30µg, sulphamethoxazole/trimethoprim (SXT) 300µg, Cefoxitin 30µg (FOX) and Tetracyclin 30mg (Thermo Scientific™ Oxoid, UK). (CLSI, 2015).

Statistical analysis

Demographic, clinical and laboratory data of study subjects were analyzed using SPSS program for windows version 20.0 (IBM, USA). Categorical variables were analyzed using the chi-square test. P-values of < 0.05 were considered statistically significant.

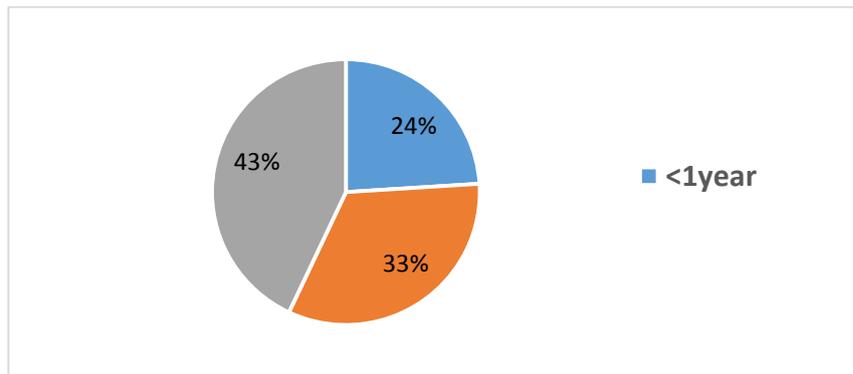
Result

Study population:

Out of 100 *E. coli* isolates, 24% of the cases aged <1 year, 33% of them between 1-2 years old and 43% of them aged between 2-5 years figure (1). Fifty-two (52%) of the cases pass more than 3 times of loose stools daily, while only 48% pass more than 5 times daily. Presence of blood and mucus was reported in 16% & 69% respectively 14% of cases has hemolytic activity. Vomiting was detected with a frequency of 58% of cases. In current study, in cases with **age <1 year**, the frequency of clinical manifestations was as the following; mucous in stool (70.8%), diarrhea > 3 daily (41.7%), diarrhea > 5 daily (58.3%), vomiting (54.2%), blood (20.08%) and hemolytic activity (25%) (table1) In cases with **age group between 1-2 years**, the majority of cases have mucous in stool and vomiting (60.6%) for each, while Diarrhea > 3, Diarrhea > 5, blood and hemolytic activity was presented in 51.5%, 48.5%, 18.2% & 9.1% respectively. In cases with **age group between 2-5 years**, the majority of cases have mucous in stool (74.4%), Diarrhea > 3, Diarrhea > 5, vomiting, Blood and Hemolytic activity was presented in 58.1%, 41.9%, 11.6% & 11.6% respectively (table 2). There was **non-significant difference** in clinical presentation (diarrhea, vomiting, blood, mucous & hemolytic activity) among age groups (<1, 1-2 and 2-5 years) as p= 0.432, 0.432, 0.888, 0.564, 0.424 & 0.231 respectively.

Antimicrobial susceptibility of the studied cases

As regards to antibiotic response, (89%) of cases was highly sensitive to Meropenem, while (61% & 59%) was sensitive to Amikacin & Cefoxitin respectively. On the other hands 52%, 65%, 75%, 60% & 70. Was resistant to trimethoprim - Sulfamethoxazole, Ceftriaxone, Tetracycline, Ceftazidime & Amoxicillin-clavulanate respectively (table3).

Figure (1): distribution of age groups among studied samples .**Table (1):** Distribution of symptoms among studied samples.

		frequency	percent
Frequency of diarrhea	More than 3 times	52	52
	More than 5 times	48	48
Presence of Vomiting		58	58
Presence of blood		16	16
Presence of mucus		69	69
Presence of Hemolytic activity		14	14

Table (2): correlation between age and clinical manifestations.

	Age group			p-value
	<1	<2	<5	
N	24	33	43	
Diarrhea > 3	10(41.7%)	17(51.5%)	25(58.1%)	0.432
Diarrhea > 5	14(58.3%)	16(48.5%)	18(41.9%)	0.432
vomiting	13(54.2%)	20(60.6%)	25(58.1%)	0.888
Blood	5(20.08%)	6(18.2%)	5(11.6%)	0.564
Mucus	17(70.8%)	20(60.6%)	32(74.4%)	0.424
Hemolytic activity	6(25%)	3(9.1%)	5(11.6%)	0.231

Table (3): Antimicrobial susceptibility of the studied cases .

	Sensitive (N/%)	Intermediate (N/%)	Resistant (N/%)
Meropenem (MEM)	89 (89%)	4(4%)	7(7%)
Amikacin (AK)	61 (61%)	18(18%)	21(21%)
Cefoxitin (FOX)	59(59%)	12(12%)	29(29%)
trimethoprim-sulphamethoxazole (SXT)	27(27%)	21(21%)	52(52%)
Ceftriaxone (CRO)	19(19%)	16(16%)	65(65%)
Tetracyclin (TET)	17(17%)	8(8%)	75(75%)
Ceftazidime (CAZ)	12(12%)	28(28%)	60(60%)
Amoxicillin-clavulanate (AMC)	12(12%)	18(18%)	70(70%)

Discussion

In the current study, out of 100 *E. coli* isolates, 24% of the cases aged <1 year, 33% of them

between 1-2 years age and (43%) of them aged between 2-5 years that agrees with Zhou et al., where (52.0%) were less than 2 years old and

48% were between 2-5 years old (Zhou et al., 2018). In a previous prospective, cross-sectional active surveillance study enrolled children which include children from 15 days to 11 years old with diarrhea and/or vomiting, the majority of cases (Eighty percent, 165 of 206) were <5 years old, and 54% were <1 year old (Foster et al., 2015).

Regarding clinical manifestations, about 52% of the cases developed more than 3 times of loose stools, while only 48% developed more than 5 times of diarrhea. Presence of blood and mucous was reported in 16% & 69% respectively & 14% of cases has hemolytic activity. Vomiting was detected with a frequency of (58%). However, Peirano et al., 2018 who examined stool from children up to 5 years of age who suffered acute community diarrhea, have recorded that ongoing diarrhea was watery in mucoid in 26 (31.32%), and blood-stained in five (6.02%) (Peirano et al., 2018). In a study performed by Zhou et al., 2018, (25.9%) of the admitted children with DEC-diarrhea developed vomiting, (11.1%) developed abdominal pain and (7.4%) developed bloody diarrhea (Zhou et al., 2018).

Conclusion

The study analyzed DEC types and their characters in Minia locality. The study also demonstrated the importance of DEC strains, particularly EAEC and EPEC that might contribute to the burden of diarrheal diseases in different age groups of children in Minia. However, we, advice to include improved detection methods for bacterial diarrheal pathogens, and appropriate studies of antimicrobial resistance in order to best manage of acute diarrhea in this region and beyond. The current study highlights the necessity for continuous monitoring of antibiotic resistance in diarrhea related bacterial pathogens. It is recommended that, in hospital and private laboratories, all diarrheal stool samples for *E. coli* should be routinely diagnosed.

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