Egyptian Journal of Medical Microbiology Volume 31 / No.3 / July 2022 83-90 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Study of Enteropathogenic *Escherichia coli* producing Extended Spectrum β-lactamase in Children with Acute Gastroenteritis

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Key words: ***Enteropathogenic E coli (EPEC), Extended-spectrum β-lactamase (ESBL), Acute gastroenteritis***

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ABSTRACT

Background*: Pathogenic Escherichia coli (E. coli) represents an important etiology of acute gastroenteritis in children. Among the pathogenic E.coli, enteropathogenic E.coli (EPEC) is associated with acute gastroenteritis in children.* ***Objectives:*** *The present study aims to detect the presence of t typical EPEC and atypical EPEC in children with community acquired diarrhea in age ≤ 5 years old by polymerase chain reaction for eae, bfp, Stx genes Also, to determine the presence of ESBL by phenotypic method and by polymerase chain reaction for TEM, PER, CTX-M genes.* ***Methodology:*** *The isolated E.coli were subjected to antimicrobial susceptibility test by the disc diffusion method according to clinical laboratory standard guidelines (CLSI) . E.coli with resistance to cefotaxime were examined for the presence of ESBL by the double discs method. Polymerase Chain Reaction was done to detect EPEC; eae, bfp, Stx genes and ESBL; TEM, PER, CTX-M genes.* ***Results:*** *A total of 290 isolates of E. coli were isolated from children with community acquired diarrhea, EPEC was identified by the presence of eaeA gene in 115 isolates of E.coli. Typical EPEC with eaeA+, bfp +, stx – genotype profile was detected in 81 (70.4%) of EPEC and atypical EPEC with eaeA+, bfp –, stx – genotype profile was detected in 34 (29.6%) of EPEC isolates. The most frequent detected ESBL genes in EPEC were CTX-M (46.9%) followed by PER (11.3%) and TEM (9.6%.). There was a significant increase in resistance to ampicillin, cefuroxime, cefotaxime, ciprofloxacin, gentamicin, Trimethoprim/sulfamethoxazole, amikacin in EPEC compared to non EPEC. Moreover, there was a significant increase in ESBL in EPEC (P=0.0001) compared to non EPEC isolates.* ***Conclusion****: The current study highlights the presence of EPEC as a common pathogen in children with acute gastroenteritis. The typical EPEC was more common than atypical EPEC genotype. The ESBL was significantly associated with EPEC with the common gene CTX-M. Knowledge of antimicrobial resistance of EPEC and other diarrheagenic E.coli is important in selecting the appropriate therapy in serious diarrheagenic Escherichia coli infections and performing local antimicrobial guidelines.*

INTRODUCTION

Acute diarrhea associated with acute gastroenteritis represents a major health problem in children below five years and it is considered as the second cause of mortality in children after pneumonia. The estimated annual death due to diarrhea in children is around 525000 [1,2].

Prolonged diarrhea can lead to dehydration with loss of body fluids and electrolytes, and this can lead to severe complications such as acute renal failure in infants and young children[3,4]. Dehydration can be diagnosed by clinical examination and history of diarrhea. The use of Modified Vesikari Score (MVS) for the dehydration scale is an effective score parameter. [5,6].

Enteropathogenic *Escherichia coli (EPEC)* represents a common bacterial pathogen associated with moderate to severe diarrhea, watery non bloody diarrhea in children; can lead to malnutrition; and cause death, particularly in children under 12 months [7,8] .

The pathogenicity of EPEC is attributed to the carrying of various genes leading to attaching effacing lesions (A/E) lesion with destruction of the brush border of the intestinal microvilli with intimate attachment to the intestinal epithelium cells and formation of pedestal structures at the adhesion site of the bacteria [9].

The genes which are responsible for A/E lesion formation are located on the enterocyte effacement locus, this locus encodes the (intimin) adhesion by the *eae* gene [10].

Enteropathogenic *Escherichia coli* and Shiga-toxin producing *E. coli* (STEC) could produce A/E lesion by *eaeA* gene, but shiga toxin encoding gene (*stx-1, stx-2*) is present only in STEC, which is used for distinguishing between these pathotypes [11].

Furthermore, EPEC had a plasmid that is known as EPEC adherence factor plasmid (pEAF) encoding type 1V pilus (bundle-forming pilus) by the *bfp* gene. The adherence factor leads to the adherence of *E.coli* to the epithelium cells of the intestine with the formation of the microcolony [12].

The *eae* (intimin) and *bfp*A genes are useful for identifying EPEC and for subdividing this group of bacteria into typical and atypical strains [10].

There are two distinct types of EPEC according to the presence of EAF plasmid, the typical EPEC (tEPEC) and atypical EPEC (aEPEC) with missing of this plasmid. The genotype profile of the typical EPEC is the presence of (*eaeA +, bfp +, stx* -), while the aEPEC had the genotype profile (*eae A+, bfp -, stx* -) with absence of *bfp* virulence factor

The tEPEC is responsible for diarrhea in most of the studies, while there is a converse about the role of aEPEC as a pathogen associated with diarrhea in children [13-15].

The improper use of antibiotics in treating mild diarrhea, especially in the developing world where the rate of diarrheal diseases is the highest and the use of antimicrobial agents is often indiscriminate, can lead to rise in the magnitude of antimicrobial resistance [14].

The presence of extended spectrum beta-lactamase (ESBL) resistance among EPEC represents a major concern in these isolates, especially with the migration of wide spread of ESBL among Enterobacteriaceae [16]. ESBL producing strains often show multidrug resistance, limiting the therapeutic options. The responsible genes for the ESBL activity are *TEM, CTX, SHV, PER,* and *OXA* [17, 18].

The present study aims to detect the presence of tEPEC and aEPEC in children with community acquired diarrhea in age ≤ 5 years old by polymerase chain reaction for *eae, bfp, Stx* genes Also, to determine the presence of ESBL by phenotypic method and by polymerase chain reaction for *TEM, PER, CTX-M* genes

METHODOLOGY

The study was a retrograde cross-sectional study that included 700 children below 5 years with acute gastroenteritis recruited from Out-patient Clinics from Mansoura University Children Hospital, Egypt, from January 2021 till February 2022. The inclusion criteria in the study were children ≤5 years with clinical manifestations of diarrhea defined by WHO as three times or more of loose or watery diarrhea within 24 hours with or without vomiting.

Children with gastrointestinal manifestations due to other etiologies such as drug reaction, renal diseases or liver disorders, and parasitic infection were excluded from the study. The study was approved by Mansoura Faculty of Medicine Ethical Committee (R **22.3.1661),** and consent approval was obtained from their parents.

The included children were subjected to medical history taking and clinical examination. The presence of dehydration and its score was recorded according to the scale of Vesikari [19]. The dehydration was considered mild if the score was < 7, moderate 7-10, severe if the score was ≥11.

Stool Sample:

Stool samples were transported to the laboratory within 30 minutes in a clean leak proof container and were inspected for the presence of blood and/or mucus and for parasites by routine microscopy.

Microbiological Culture:

The stool sample was enriched first in MacConkey broth (Oxoid, United Kingdom) for 24 hours at 37C. Then, subcultured on MacConkey agar plate. After overnight incubation at 37°C, lactose fermenting colonies on MacConkey agar were identified by standard laboratory techniques for the identification of *E. coli*. *E. coli* strain ATCC 25922 was used as a positive control.

Antimicrobial susceptibility testing:

E.coli isolates were subjected to antimicrobial susceptibility test by disc diffusion method according to clinical laboratory standard guidelines CLSI guidelines[20]. Antimicrobial discs used were: Ampicillin (10µg), Cefuroxime (30µg), Amoxicillin-Clavulanic acid (30µg), Cefotaxime (30µg), Ceftazidime (30µg) Gentamicin (30µg), Sulphamethoxazole/ trimethoprim (25µg), Ciprofloxacin (5µg), Amikacin (30µg) Imipenem (10µg) (Thermofisher-UK). *E.coli* ATCC 25922 was used as a quality control strain

Phenotypic Detection of ESBL Producers:

The isolated *E.coli* with resistance to ceftazidime and/or cefotaxime were studied for the presence of ESBL by the double disc diffusion method using cefotaxime (30 mg µg at a distance of 20 mm from a disk containing cefotaxime/clavulanic acid (30/10 µg)

The increase in the sensitivity zone diameter of cefotaxime/clavulanic acid disk by ≥ 5mm was considered as an indicator of ESBL production according to CLSI [20]. The. *Escherichia coli* ATCC25922 was used as negative control and *Klebsiella pneumoniae* ATCC700603 was as positive control for ESBL production.

Polymerase Chain Reaction for EPEC and ESBL genes:

- ***DNA Extraction of E.coli.***

Two colonies of *E.coli* were suspended in one milliliter of distilled water and incubated at 95ºC for 10 minutes. Then the suspension was centrifuged for 5 minutes at 1,000 rpm. The supernatant was decanted

and the pellet was stored at -20ºC until use for further DNA analysis [21].

- ***PCR for detection of B-lactamase genes***

The sequences of the used primers for the amplification of *B-lactamase* genes (*blaTEM, blaSHV, blaCTX-M*) by PCR were summarized in table 1 [21].For the amplification procedures we used 3 microns of the extracted DNA applied over 25 microns of ready to use master mix with 0.2 microns of Taq polymerase 5 U/μl (Qiagen) with l μl of each) from each reverse and forward primer. [21]

- ***PCR for Identification of EPEC***

Detection of *eaeA, bfp, stx-1,* and *stx-2* genes were determined by multiplex PCR. Ready to use amplification kit was purchased from Qiagen (Qiagen-Germany).

The amplification conditions were as follows: Initial denaturation at 94ºC for 3minutes; 35 cycles of 94ºC for 45 seconds, annealing as found in table 1 for 45 seconds, extension at 72ºC for 1 minute, and a final extension at 72ºC for 5 minutes. PCR products were subjected to electrophoresis with 1.5% agarose gel with 0.5µg/mL ethidium bromide and visualized using ultraviolet light.

The EPEC isolates were defined as *eaeA*-positive and *stx*-negative, typical EPEC were defined as (*eaeA+, bfp +, stx -)*, while the aEPEC had the genotype profile (*eae A+, bfp -, stx -*) with absence of bfp virulence factor [22].

Table 1: Primers used for PCR amplification of the studied genes

Gene	Primer Sequence	Base pair	Reference
CTX -M	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550	21
TEM	GAGTATTCAACATTTCCGTGTC TAATCAGTGAGGCACCTATCTC	810	21
PER	AATTTGGGCTTAGGGCAGAA ATGAATGTCATTATAAAAGC	925	21
eaeA	CATTATGGAACGGCAGAGGT ATCTTCTGCGTACTGCGTTCA	790	22
bfpA	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	326	22
Stx -1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	22
Stx-2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	22

Statistical Analysis

The data of the study was analyzed by the use of SPPS 22. The numerical data was expressed as median, minimum, and maximum if it is non-parametrical data. The qualitative data was expressed as number and percentage and the comparison was performed by chi-square and P is considered significant >0.05.

RESULTS

The study included 290 isolates of *E. coli* isolated from children with community acquired diarrhea. The median age of the children was 29.00 months with minimum age 1.00 month and maximum age 60 months. They were 165 (56.9%) males and 125 (43.1%) females with urban residence in 51% and urban residence in 49%.

Besides diarrhea, the major symptoms were fever in 139 (47.9%), followed by abdominal pain (39.3%) and vomiting (37.9%). The dehydration was mild to moderate (43.4%, 48.3% respectively). While the minority of the children had severe dehydration (8.3%) according to Vesikari score (table 2).

Table 2: Demographic and clinical data of the studied children

Age (Months)	
Median	29.00
Minimum	1.00
Maximum	60.00
Sex	
Male	165 (56.9%)
Female	125 (43.1%)
Abdominal pain	114 (39.3%)
Fever	139 (47.9%)
Vomiting	110 (37.9%)
Vesikari score	
1.00	126 (43.4%)
2.00	140 (48.3%)
3.00	24 (8.3%)
Residence	
Rural	142 (49%)
Urban	148 (51%)

ESBL Detection by phenotypic double disc method was positive in 126 out of 290 (43.4%) of the isolated *E.coli.* Distribution of *TEM, PER,* and *CTX-M* genes among ESBL producing *E.coli* were as follows 12.7%, 18.2%, and 63.5%; Respectively (table 3).

Table 3: Distribution of *TEM, PER and CTX-M* genes among E.*coli* ESBL producers.

	ESBL (n=126)	
	No.	%
TEM	16	12.7
Per	23	18.2
CTX-M	80	63.5

EPEC was identified by the presence of *eaeA* gene in 115 (39.6%) isolates of *E. coli*. Typical EPEC with *eaeA+*, *bfp +*, *stx* – genotype profile was detected in 81 (70.4%) of EPEC and atypical EPEC with *eaeA+*, *bfp –*, *stx –* genotype profile was detected in 34 (29.6%) of EPEC (table 4).

Table 4: Typical and atypical EPEC among EPEC determined by PCR

	EPEC (n=115)	
	No.	%
Typical EPEC	81	70.4
Atypical EPEC	34	29.6

Regarding ESBL genes, there was significant increase in *CTX-M* (P=0.0001) and *TEM* gene (P=0.0.15) in EPEC compared to non EPEC (table 5).

Table 5: ESBL genes detected in EPEC and non EPEC isolates

	EPEC (n=115)		Non-EPEC (=175)		P
	N0.	%	N0.	%	
CTX-M	54	46.95	26	14.9	0.0001
TEM	11	9.6	5	2.9	0.015
PER	13	11.3	10	5.7	0.07

In comparison between children with EPEC and children with non EPEC, there was insignificant difference in sex, residence, abdominal pain, fever, vomiting and the severity of dehydration (P=0.3, P=0.4, P=0.8, P=0.4, P=0.23, P=0.9 respectively). However, EPEC was significantly isolated from children with age ≤ 2 years (58.3%) compared to children with non EPEC (39.4%, P=0.002) (table 6).

Table 6: Comparison between clinical and demographic data of children with EPEC and children with non EPEC isolates

	EPEC (n=115)		Non-EPEC (=175)		P	95%CI	Odds Ratio
	N0.	%	N0.	%			
Sex							
Male	63	54.8	102	58.3	0.3	0.87	0.54-1.39
Female	52	45.2	73	41.7			
Age							
≤ 2 years	67	58.3	69	39.4	0.002*	2.1	1.3-3.46
>2 years-5	48	41.7	106	60.6			
Rural	58	50.4	84	48	0.4	1.1	0.69-1.78
Urban	57	49.6	91	52			
Abdominal pain	39	33.9	75	42.9	0.08	0.684	0.42-1.11
Fever	57	49.6	82	46.9	0.4	1.1	0.69-1.78
Vomiting	47	40.9	63	36.0	0.23	1.2	0.76-1.9
Vesikari score							
1	41	35.7	85	48.6		-	-
2	63	54.8	77	44.0	0.09		
3	11	9.6	13	7.4			

There was significant increase in resistance to ampicillin, cefuroxime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, Trimethoprim/ sulfamethoxazole, amikacin in EPEC (P=0.0001, P=0.0001, P=0.0001, P=0.001, P=0.0001, P=0.002, P=0.001, P=0.0001, respectively) compared to non EPEC. Moreover, there was significant increase in ESBL in EPEC (P=0.0001) compared to non EPEC isolates (table 7).

Table (7): Antimicrobial resistance patterns among EPEC and non- EPEC

Antibiotic	EPEC (n=115)		Non-EPEC (=175)		P
	N0.	%	N0.	%	
Ampicillin	85	73.9	50	28.5	0.0001
Cefuroxime	87	75.6	54	30.9	0.0001
Amoxicillin/clavulanic acid	82	71.3	42	24	0.2
Cefotaxime	85	73.9	45	25.7	0.0001
Ceftazidime	85	73.9	53	30.3	0.0001
Ciprofloxacin	43	37.4	31	17.7	0.0001
Gentamicin	55	47.8	49	28	0.002
Trimethoprim/sulfamethoxazole	77	44	49	28	0.0001
Amikacin	54	46.95	44	25.1	0.001
Imipenem	0	0	0	0	0.9
ESBL	83	72.2	43	24.6	0.0001

*ESBL: Extended-spectrum β-lactamase; ***p-value*** ≤ 0.05 is statistically ***significant***

DISCUSSION

Enteropathogenic *Escherichia coli* (EPEC) is a major etiology of acute diarrhea in children below 5 years old, due to its high prevalence in hospital and community settings, it is responsible for approximately 11% of all diarrhea mortalities in children aged below five years in the world [23].

Clinical diagnosis of EPEC has limited value as the symptoms associated with this pathogen are similar to acute gastroenteritis associated with other pathogens. Therefore, the diagnostic microbiology laboratory has the major role in detection of the responsible pathogen for this infection [24].

In the present study, the clinical symptoms in children with *E.coli* were diarrhea, fever, vomiting and abdominal pain with statistically insignificant differences between children with EPEC and those without EPEC.

The symptoms of acute gastroenteritis may indicate the affected part of the gastrointestinal tract as vomiting is usually associated with the upper intestine, severe abdominal pain may indicate affection of the large intestine and fever suggests the presence of inflammation with tissue invasion and dehydration [25, 26].

The majority of the affected children with EPEC in the present study had moderate dehydration according to Vesikari score. Children with diarrhea are liable to dehydration due to the loss of fluid with electrolytes. The diagnosis of dehydration level is required for appropriate fluid resuscitation either oral or intravenous [24]. Dehydration is one of the clinical features of EPEC diarrhea [27].

In the present study, 115 *E.coli* isolates out of 290 (39.6%) were defined as EPEC by the presence of *eaeA* gene by PCR. The prevalence of EPEC association with diarrhea in children ranged from 5.6% up to 44.9% [28-32]. In Egypt, a recent study by Khairy et al. [18] reported that EPEC prevalence was 28.8% among studied children.

The difference in the prevalence rates of EPEC in children can be attributed to various reasons such as the difference in the age of the included children, the difference in the geographical regions, the difference of the laboratory methods used to identify EPEC and the difference in the antibiotics prescription policy [33,34].

In the present study, EPEC had a significantly higher prevalence in children with age ≤ 2 years old. Similar finding was reported previously [27].

In previous reports, the major type of EPEC detected associated with diarrhea was the typical variant [35]. Nevertheless, the atypical variant had emerged as a frequent pathogen detected in diarrhea [21,45.] In the Current study, typical EPEC was the common variant (70.4%) of EPEC and atypical EPEC represents 29.6% . In agreement with our findings, Similar results were reported in earlier studies [18,32.]

In the current study, EPEC had significantly high resistance to cefotaxime, ceftazidime, amikacin, gentamicin, Sulfamethoxazole, this is in accordance with other studies [32,36-38].

Phenotypic testing of ESBL of isolated *E. coli* showed that 126 isolates (43.4%) were ESBL producers. This finding is in accordance with the high proportion of ESBL reported in previous studies [22,39] Moreover, there was significant increase in ESBL in EPEC (P=0.0001) compared to non EPEC isolates. This may be due to overuse of antibiotics for the treatment of mild diarrheal cases. Fortunately, All EPEC isolates were susceptible to imipenem, which is in congruence with other studies [21,36]

In our study, the most frequently detected ESBL gene in EPEC was *CTX-M* (46.9%) followed by *PER* (11.3%) and *TEM* (9.6%.). Similar results were reported by previous study [40]. on contrary to our results, study performed in India by Singh et al [30], found that *TEM* was the most detected gene.

The high prevalence of ESBL in EPEC is a major concern that limits the therapeutic option in severe cases which requires antibiotics therapy. This finding supports

the requirement for active surveillance of antibiotic resistance pathogens in community acquired gastroenteritis and the requirement for a strict antibiotic prescription policy.

CONCLUSION

The current study highlights the presence of EPEC as a common pathogen in children with acute gastroenteritis. The typical EPEC was more common than atypical EPEC genotype. The ESBL was significantly associated with EPEC with the common gene *CTX-M*. Knowledge of antimicrobial resistance of EPEC and other diarrheagenic *E.coli* is important in selecting the correct antibiotic therapy in serious diarrheagenic infections and for performing local antimicrobial guidelines.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

REFERENCE

1. Zaeni IA, Ratri DP, Taufani AR. Classification of the Degree of Dehydration in Diarrhea Using the Naïve Bayes Algorithm. 2021 IEEE International Biomedical Instrumentation *and Technology Conference* (IBITeC*)*, 2021, pp. 148-152
2. World Health Organization. *Diarrhoeal disease*. 2016
3. Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ. Black RE Organization Child Health Epidemiology Reference Group of the World Health, and Unicef. Global causes of diarrheal disease mortality in children< 5 years of age: a systematic review. PLoS One. 2013;8: e72788.
4. Passariello A, Terrin G, Baldassarre ME, De Curtis M, Paludetto R, Canani RB. Diarrhea in neonatal intensive care unit. World Journal of Gastroenterology: WJG. 2010 Jun 7;16(21):2664.
5. Deacon V, Dziva F, Van Diemen PM, Frankel G, Stevens MP. Efa-1/LifA mediates intestinal colonization of calves by enterohaemorrhagic *Escherichia coli* O26: H–in a manner independent of glycosyltransferase and cysteine protease motifs or effects on type III secretion. Microbiology. 2010;156(8):2527-36.
6. Cassady-Cain RL, Blackburn EA, Alsarraf H, Dedic E, Bease AG, Böttcher B, Jørgensen R, Wear M, Stevens MP. Biophysical characterization and activity of lymphostatin, a multifunctional virulence factor of attaching and effacing *Escherichia coli*. Journal of Biological Chemistry. 2016 ;291(11):5803-16.
7. Pawlowski SW, Warren CA, Guerrant R. Diagnosis and treatment of acute or persistent diarrhea. Gastroenterology. 2009 ;136(6):1874-86.
8. Abba K, Sinfield R, Hart CA, Garner P. Pathogens associated with persistent diarrhoea in children in low and middle income countries: systematic review. BMC infectious diseases. 2009 ;9(1):1-5.
9. Lai Y, Rosenshine I, Leong JM, Frankel G. Intimate host attachment: enteropathogenic and enterohaemorrhagic *E scherichia coli*. Cellular microbiology. 2013;15(11):1796-808.
10. Franzin FM, Sircili MP. Locus of enterocyte effacement: a pathogenicity island involved in the virulence of enteropathogenic and enterohemorragic *Escherichia coli* subjected to a complex network of gene regulation. BioMed research international. 2015; (534738)
11. Cepeda-Molero M, Berger CN, Walsham AD, Ellis SJ, Wemyss-Holden S, Schüller S, Frankel G, Fernández LÁ. Attaching and effacing (A/E) lesion formation by enteropathogenic *E. coli* on human intestinal mucosa is dependent on non-LEE effectors. PLoS pathogens. 2017;13(10): e1006706.
12. Teixeira NB, Rojas TC, da Silveira WD, Matheus-Guimarães C, Silva NP, Scaletsky IC. Genetic analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor (EAF) plasmid reveals a new deletion within the EAF probe sequence among O119 typical EPEC strains. BMC microbiology. 2015;15(1):1-9.
13. Hu J, Torres AG. Enteropathogenic *Escherichia coli*: foe or innocent bystander? *Clin Microbiol Infect*. 2015;21(8):729-34.
14. Abreu AG, Bueris V, Porangaba TM, Sircili MP, Navarro-Garcia F, Elias WP. Autotransporter protein-encoding genes of diarrheagenic *Escherichia coli* are found in both typical and atypical enteropathogenic *E. coli* strains. *Appl Environ Microbiol*. 2013;79(1):411-4
15. Botkin DJ, Galli L, Sankarapani V, Soler M, Rivas M, Torres AG. Development of a multiplex PCR assay for detection of Shiga toxin-producing *Escherichia coli*, enterohemorrhagic *E. coli*, and enteropathogenic *E. coli* strains. *Front Cell Infect Microbiol*. 2012; 2:8

16. Flokas ME, Karanika S, Alevizakos M, Mylonakis E. Prevalence of ESBL producing Enterobacteriaceae in pediatric bloodstream infections: a systematic review and meta-analysis. *PLoS One*. 2017;12(1).
17. Xu Y, Sun H, Bai X, Fu S, Fan R, Xiong Y. Occurrence of multidrug-resistant and ESBL-producing atypical enteropathogenic *Escherichia coli* in China. *Gut Pathog*. 2018; :8.
18. Khairy RMM, Fathy ZA, Mahrous DM, Mohamed ES, Abdelrahim SS. Prevalence, phylogeny, and antimicrobial resistance of *Escherichia coli* pathotypes isolated from children less than 5 years old with community acquired- diarrhea in Upper Egypt. BMC Infect Dis. 2020;20(1):908
19. Schnadower D, Tarr PI, Gorelick MH, O'Connell K, Roskind CG, Powell EC, Rao J, Bhatt S, Freedman SB. Validation of the modified Vesikari score in children with gastroenteritis in 5 US emergency departments. J Pediatr Gastroenterol Nutr. 2013 ;57(4):514-9.
20. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
21. Gangoué-Piéboji J, Bedenic B, Koulla-Shiro S, Randegger C, Adiogo D, Ngassam P, Ndumbe P, Hächler H. Extended-spectrum-β-lactamase-producing Enterobacteriaceae in Yaounde, Cameroon. Journal of clinical microbiology. 2005 Jul;43(7):3273-7.
22. Sirous M, Hashemzadeh M , Keshtvarz M , Amin M, Shams N. Molecular Characterization and Antimicrobial Resistance of Enteropathogenic *Escherichia coli* in Children from Ahvaz, Iran. Jundishapur J Microbiol. 2020;13(7): e100877.
23. Saka HK, Dabo NT, Muhammad B, García-Soto S, Ugarte-Ruiz M, Alvarez J. Diarrheagenic *Escherichia coli* pathotypes from children younger than 5 years in Kano State, Nigeria. Frontiers in Public Health. 2019:348.
24. Rivera-Dominguez G, Ward R. Pediatric Gastroenteritis In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2021.
25. Lo Vecchio A, Buccigrossi V, Fedele MC, Guarino A. Acute Infectious Diarrhea. Adv Exp Med Biol. 2019; 1125:109-120
26. Raval MV, Kwan AB, Travers CD, Heiss KF. Importance of Compliance Audits for a Pediatric Complicated Appendicitis Clinical Practice Guideline. J Med Syst. 2018 Nov 07;42(12):257
27. Tilak GP, Mudaliar JL. Role of enteropathogenic *Escherichia coli* in paediatric diarrhoeas in South India. Mater Sociomed. 2012;24(3):178-81
28. Behiry IK, Abada EA, Ahmed EA, Labeeb RS. Enteropathogenic *Escherichia coli* associated with diarrhea in children in Cairo, Egypt. *ScientificWorldJournal*. 2011; 11:2613-2619.
29. Nakhjavani FA, Emaneini M, Hosseini H, Iman-Eini H, Aligholi M, Jabalameli F, Haghi-Ashtiani MT, Taherikalani M, Mirsalehian A. Molecular analysis of typical and atypical enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhoea. Journal of medical microbiology. 2013 ;62(2):191-5.
30. Singh T, Das S, Ramachandran VG, Wani S, Shah D, Maroof KA, Sharma A. Distribution of integrons and phylogenetic groups among enteropathogenic *Escherichia coli* isolates from children< 5 years of age in Delhi, India. Frontiers in microbiology. 2017;8:561.
31. Amisano G, Fornasero S, Migliaretti G, Caramello S, Tarasco V, Savino F. Diarrheagenic *Escherichia coli* in acute gastroenteritis in infants in north-West Italy. New Microbiol. 2011; 34:45–51
32. Snehaa, K., Singh, T., Dar, S.A., Haque, S., Ramachandran, V.G., Saha, R., Shah, D. and Das, S., 2021. Typical and atypical enteropathogenic *Escherichia coli* in children with acute diarrhoea: changing trend in East Delhi. *biomedical journal*, *44*(4), pp.471-478.
33. Foster MA, Iqbal J, Zhang C, McHenry R, Cleveland BE, Romero-Herazo Y, Fonnesbeck C, Payne DC, Chappell JD, Halasa N, Gómez-Duarte OG. Enteropathogenic and enteroaggregative *E. coli* in stools of children with acute gastroenteritis in Davidson County, Tennessee. Diagnostic microbiology and infectious disease. 2015 ;83(3):319-24.
34. Sarantuya J, Nishi J, Wakimoto N, Erdene S, Nataro JP, Sheikh J, et al. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among *E. coli* strains causing diarrhea in Mongolian children. J Clin Microbiol. 2004; 42:133–9.
35. Buss SN, Leber A, Chapin K, et al. Multicenter evaluation of the bio fire film Array gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol. 2015; :915–25.
36. Malvi S, Appannanavar S, Mohan B, Kaur H, Gautam N, Bharti B, Kumar Y, Taneja N. Comparative analysis of virulence determinants, antibiotic susceptibility patterns and serogrouping of atypical enteropathogenic *Escherichia coli* versus typical enteropathogenic *E. coli* in India.

Journal of medical microbiology. 2015 ;64(10):1208-15.

37. Ibrahim OM, Saber-Ayad MA. Antibiotic misuse in different hospital wards (a pilot study in an Egyptian hospital). Asian J Pharm Clin Res. 2012;5(2):95-7.
38. Shahraki Zahedani S, sayadzai N. Frequency and Antibiotic Resistance Pattern of Diarrheagenic *Escherichia coli* (DEC) Strains Isolated from Children Aged Less Than 10 Years. mljgoums. 2018; 12 (2) :7-12
39. Ouchar Mahamat O, Tidjani A, Lounnas M, Hide M, Benavides J, Somasse C, Ouedraogo AS, Sanou S, Carrière C, Bañuls AL, Jean-Pierre H. Fecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae in hospital and community settings in Chad. Antimicrobial Resistance & Infection Control. 2019;8(1):1-7.
40. Mandal A, Sengupta A, Kumar A, Singh UK, Jaiswal AK, Das P, Das S. Molecular epidemiology of extended-spectrum β-lactamase–producing *Escherichia coli* pathotypes in diarrheal children from low socioeconomic status communities in Bihar, India: emergence of the CTX-M Type. Infectious Diseases: Research and Treatment. 2017 6; 10:1-11.