Isolation and characterization of enteroaggregative *Escherichia coli* among the causes of bacterial diarrhea in children

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Abstract

Diarrhea is a public health problem and an important cause of morbidity and mortality, enteroaggregative *Escherichia coli* (EAEC) have been recognized increasingly as agents of diarrhea in developing and in industrial countries. So the aim of this study is to study the prevalence of EAEC, its virulence factors and study it´s resistance to antibiotics. Results showed that *E. coli* represent 86 (32.9%) of the isolated bacteria. the high percentage 60% were EAEC, plasmid-encoded toxin (pet) was the marker most prevalent detected 14/30(28%) than EAST1(for EaggEC heat-stable enterotoxin 1)which detected in 9/30(18%) of EAEC and two virulence genes (pet^{+} and $EAST1^{+}$) were found in7/30(14 %) of EAEC. 10% of isolates were contained capsule but only 3.3%isolates of *E. coli* have Type I pili while all the isolates possess Type III pili. The results in the present study indicated that all *E. coli* were negative to Congo red dye agar test in contrast high percent of *E. coli* isolates were able to produce biofilm (86.7%) with different degree of thickness, while 20%isolates were urease positive after 24- 48 hrs, and 10% isolates were able to produce amylase. All isolates of *E. coli* were unable to produce Lecithinase, nuclease and hemolysin. While, 93.3% of *E. coli* isolates produced β-lactamase. The frequency of Sorbitol *E. coli* fermenter was 66.7%. In this study EAEC isolates were resistance (100%) to Penicillin and Ampicillin, High resistance(96.7%) was observed to each of Ceftriaxone, Cefotaxime and Trimethoprim. and Tetracycline (76.7%), resistance percentage of Both Amikacin and Rifampicin were (40%) and (33.3%) respectively. Higher sensitivity was reported to Gentamicin (63.3%), Ciprofloxacin(60%) then Nitrofurantoin (56.7%). The data from this study draw attention to the importance of notifying diarrheal disease. The high level of antimicrobial resistance observed in our study raises a broader discussion about the indiscriminate use or misuse of antibiotics and the risks of empirical antibiotic therapy in children of a very young age.

Introduction

E. coli has a dichotomous existence; as harmless or become pathogens. Current dogma suggests that such latter strains of *E. coli* have acquired additional genetic elements, encoding specific virulence factors, which enable the organism to cause disease. The resulting clinical syndromes include extraintestinal infections and intestinal infections mediating diarrhea [1].Those strains causing intestinal infections can be divided into six separate and major categories or pathotypes viz. enteroaggregative *E. coli* (EAEC), enteroinvasive (EIEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC) and diffuse adhering *E. coli* (DAEC)[2]. EAEC has recently received increasing attention as an emerging enteric pathogen [3] Since first described in 1987, EAEC have been recognized increasingly as agents of diarrhea in developing and in industrial countries [4]. EAEC strains have been implicated in acute as well as persistent diarrhea among adults and children [5] EAEC display a characteristic aggregative or ‘‘stacked-brick’’ pattern of adherence to Hep-2 cells [1] so-called because they exhibit an aggregative pattern of adherence to Hep-2 cells [6]. Most EAEC strains harbor a 60- to 65-Mda virulence plasmid (pAA). A 1-kb fragment of pAA, referred to as the EAEC probe or CVD432, The pAA plasmid also encodes Enteroaggregative heat-stable enterotoxin 1 (EAST-1)and a 104-kDa cytotoxin designated pet. In addition to the pAA plasmid, some EAEC strains express putative virulence factors that are encoded on the chromosome [7] Pet is a serine protease autotransporter (SPATE) secreted by EAEC 042 which induces dilation of crypt openings and rounding and extrusion of enterocytes in human tissue explants . Once secreted Pet exerts its toxic effects by being internalized into host cells where it cleaves the host cytoskeletal protein spectrin [1] was shown to induce increased mucus release, exfoliation of cells, and devel-opment of crypt abscesses [8]. Nucleotide sequence analysis suggests that the toxin is a member of the autotransporter class of proteins, characterized by the presence of a conserved C-terminal domain which forms a β-barrel pore in the bacterial outer membrane and through which the mature protein is transported. The Pet toxin is highly homologous to the EspP protease of enterohemorrhagic *E. coli* and to EspC of enteropathogenic *E. coli*, an as yet cryptic protein [9] EAST-1, encoded by the *astA* gene adjacent to pet [1] EAST1 (for EaggEC heat-stable enterotoxin 1)[4] which is a 38-amino acid protein [4], Named *astA* (EaggEC heat-stable enterotoxin), represents the EASTI structural gene. EAST1 shows significant homology with the enterotoxic domain of heat-stable enterotoxin a (Sta) of enterotoxigenic *E. coil* and with guanylin, a mammalian analog of Sta. Unlike Sta, which requires six cysteines and three disulfide linkages for ful biological activity, both EAST1 and guanylin contain four cysteine residues. Based on the cGMP data and the sequence homology to Sta and guanylin, it is predicted that EAST1 stimulates the

particulate form of guanylate cyclase through the same receptor binding region as Sta and guanylin [10].
Volunteers fed EAEC strains excrete mucoid stools. The formation of a heavy mucus biofilm may contribute to EAEC diarrheagenicity and, perhaps, to its ability to cause persistent colonization and diarrhea [4]. A volunteer study has shown that oral challenge with 10^{10} CFU/ml of EAEC causes diarrheal illness. Once EAEC is ingested, it can bind to the mucosa of the small and large intestines. EAEC that is bound to the intestinal mucosa stimulates epithelial cells to produce a thick mucus layer above the intact enterocyte brush border, and EAEC elicits inflammatory mediators that produce cytotoxic effects involving the intestinal mucosa[3]. EAEC isolates release toxins that bind to the intestinal mucosa and elicit inflammatory mediators that produce cytotoxic effects and intestinal secretion [3].
Most EAEC isolates produce biofilm, which is associated with the multiple EAEC genes [3]. The genome of 042 was found to possess many genetic characteristics of pathogenic Shigella, Salmonella and diarrheagenic *E. coli* strains [1]. pet enterotoxin shows homology with members of the autotransporter family of bacterial proteins [9] Certainly, the association of most autotransporters in *E. coli* and *Shigella* with the IS*629*-like elements suggests a role for this element in the evolution and spread of these homologs among the Enterobacteriaceae [9]. Currently, identification of EAEC is not routinely performed [3] Limitations of the Hep-2 cell assay include time requirements and limited availability in reference laboratories. These limitations have led to the search for other diagnostic methods, including polymerase chain reaction (PCR) assays [3]. So the aim of this study is to study the prevalence of EAEC, its virulence factors and study it´s resistance to antibiotics.

Materials and Methods

Samples collection

This study was carried out on children (out and inpatient suffering from bacterial diarrhea) attending Tikrit teaching hospital and Baeiji hospital in Salah al din city during the period from February 2012 until February 2013. A total of (150) patients aging from 20 days to 60 months were included in this study, Stool specimens were collected from those patients and processed immediately or used Carry Blair transport media if delayed for 1-2 hours after their collection and then cultured [11].

Bacterial isolation and identification

Collected samples were cultured directly on MacConkey agar for primary isolation of the Enterobacteriaceae, and then purified on MacConkey agar and EMB agar. All isolates were incubated aerobically at 37 ˚C for 24hr, and suspicious colonies were selected for definitive microscopic examination, culture characteristics, biochemical testing and the usage of API 20E System (BioMérieux/France) for identifying *E. coli* [12]

E. coli Genomic DNA Extraction

Genomic DNA was extracted from EAEC cultures using OMEGA DNA Mini Kit according to the manufacturers ‘instructions for Protocol. The DNA concentration was measured using a ND-1000 Spectrophotometer (NanoDrop), then DNA samples of *E. coli* isolates were universe concentration about 50ng/ µl by using the formula: C1V1=C2V2.

Polymerase Chain Reaction Assay

The DNA primers were prepared depending on manufacturer instruction. *E. coli* DNA templates were subjected to PCR using 2 sets (F and R) of primers [13] targeting groups of genes listed in Table(1) to determine the virulence properties and to identify EAEC. Assembling PCR materials were done according to the procedure of Promega corporation (USA), using PCR reaction mixtures prepared in 0.2 ml eppendorf tube with 20 µl reaction volumes, which contain: 1 µl premix, 1 µl forward primer, 1 µl reverse primer, 1 µl DNA template, 16 µl nuclease-free water. All the appending was done in laminar flow on ice. The PCR amplification conditions performed with a thermal cycler were specific to each single primer set as in Table (1)

Table (1) Primers and thermo cycler program

Primer	Thermo cycling condition	DNA Sequences (5’-3’)	size	Refe.
pet	Stage 1 ×1: 95.0 5:00 Step 1; Stage 2 ×35: 95.0 0:40 Step 1, 55.0 1:00 Step 2, 72.0 0:45 Step 3; Stage 3 ×1: 72.0 7:00 Step 1, 4.0 – Step 2	F:GACCATGACCTATACCGACAGC R:CCGATTTCTCAAACTCAAGACC	599	Presterl *et al*, 2003
EAST 1	Stage 1 ×1: 95.0 5:00 Step 1; Stage 2 ×30: 95.0 0:40 Step 1, 60.0 1:00 Step 2, 72.0 0:45 Step 3; Stage 3 ×1: 72.0 10:00 Step 1, 4.0 – Step 2	F:TGCCATCAACACAGTATATCC R:TAGGATCCTCAGGTCGCGAGTGACGGC	116	

The amplified PCR products were detected by 2% agarose gel electrophoresis. PCR products were loaded to the agarose gel wells and 100 bp ladders to one of the wells in each row. The electric current was performed in two stages: first at 35 volt for 15min, second at 50 volt for 1hr. then staining with ethidium bromide. The electrophoresis result was detected by using gel documentation. Finally, the gel was photographed using gel documentation saving picture

Detection of some virulence factors

For all isolates Urease activity [14], Hemolysis [15], Binding to Congo red [16], Dnase production [17], Lecithinase production [8], starch hydrolysis [17], β-lactamase[19], capsule[20], biofilm formation[21], TypeI pili and Type III pili [22], sorbitol fermentation[15] were determined.

Antibiotic Susceptibility Testing

The antimicrobial susceptibility assay was performed on Mueller-Hinton agar by the disc-diffusion method (Kirby–Bauer) [15] and growth inhibition zones were interpreted according to the Clinical Laboratory Standards Institute [23]. The antimicrobial disks (Penicillin, ampicillin, ceftriaxone, Cefotaxime, gentamicin, amikacin, Rifampicin, Ciprofloxacin, Nitrofurantoin, tetracycline, Trimethoprim) were of commercial grade (Bioanalyse, Turkey).

Results and discussion

The results of identification showed that among the 150 cultured stool samples, 2 samples were culture negative identified while 148 samples were culture positive, And 261 bacteria isolated from children with diarrhea, *E. coli* represent 86 (32.95%) of the isolated bacteria . The male to female ratio was 1.1:1. Higher incidence of diarrhea was recorded at age group since birth to 10 months, with 67 (45%) of recorded patients.

The results of DNA isolation from 50 isolates of DEC, which were selected as representing sample, indicated that each one of *E. coli* isolate was contained chromosome and various number of small plasmid DNA bands approximately in the same size in some isolates whereas in different size in others as show in Figure(1) the plasmids because of its unique molecular weight and size, makes a band on certain position of gel surface which is distinct from that of chromosomal DNA bands as it has heavy molecular weight. The difference in plasmid profiles of DEC isolates in the present study was in agreement with that reported by Ali (2006) who showed that plasmids of EPEC isolated from human patient were distributed widely and showed great diversity in their molecular weight [24], while Todorova *et al.* (1990) showed that 92% of *E. coli* serotype O164 strain possess two small plasmids of molecular sizes 9.06kb and 7.248kb [25].

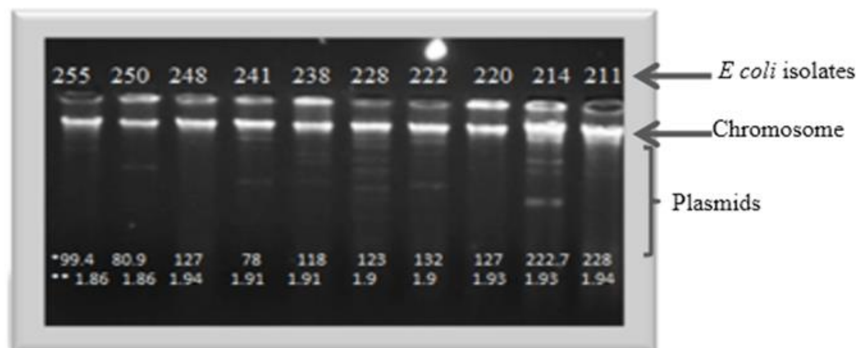

Figure (1) DNA profile of *E coli* isolates as electrophoresis on 1% agarose gel and stain with Ethidium Bromide,*concentration of DNA (ng/μl), Purity of DNA**

The results of PCR demonstrate that the high percentage (60%) of 50 DEC strains were EAEC. Many reports have demonstrated the association of EAEC with diarrhea in children in developing countries. Salih (2007) detected EAEC pathotype among 31 *E. coli* isolates, she found 18 (58.08%) strains was EAEC [26].On the other hand, the prevalence of EAEC observed in this study was marginally higher or higher compared with the studies of Cohen *et al.* (1993) found that the percentage of EAEC isolated is 52.4% [27]; Piva *et al.* (2003) also indicated that the isolation rate of EAEC was 42% [28]; Teng *et al.* (2004) in Taiwan showed that EAEC (8.7%) isolates [29] while Johnnie (2005) Who has found that the isolation rates of EAEC was 19% in India and 26% in Jamaica [30].*pet* was the marker most prevalent detected 14/30(28%) than EAST1which detected 9/30(18%) in EAEC and two virulence genes (pet^{+} and EAST1^{+}) were found in7/30(14 %) of EAEC. The occurrence of pet and EAST1in the present study was analogous to that recorded by other studies such as, Okeke *et al.* (2000) detected EAST-1 in 23% of EAEC strains [31]. In a study conducted in Brazil, Zamboni *et al.* (2004) reported that pet and EAST-1 were the most frequently detected markers 40.8% and 26.5% respectively in EAEC strains [7]. On the other hand, the prevalence of pet and EAST1 observed in this study was marginally lower compared with the studies of Rich *et al.* (1999) who detected EAST-1 in 45% of EAEC strains [32]. In a study conducted in Brazil, Piva *et al.* (2003) detected EAST-1 in 73% of EAEC strain [28].

Table (2) percentage of *E. coli* groups and genes

E. coli group	No. positive (%)	Primer
EAEC	30/50(60%)	Pet 14/30(28%) EAST1 9/30(18%) Pet+EAST1 7/30(14%)

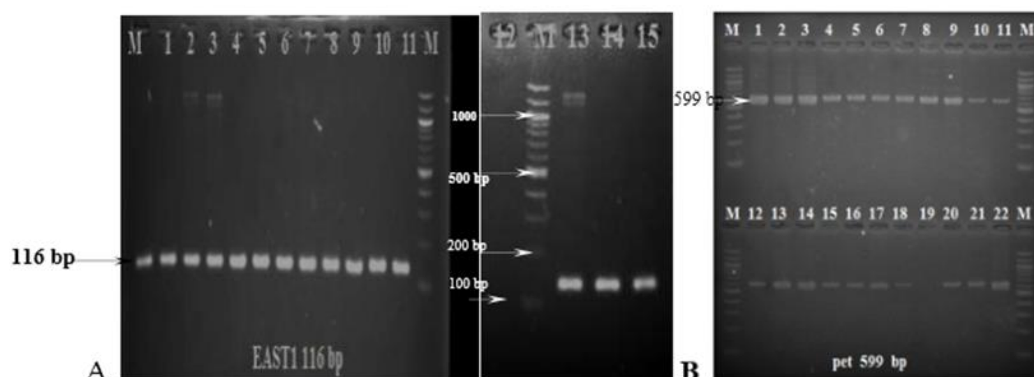

Figure (2) Ethidium bromide-stained 2%agarose gel of PCR amplified products from extracted *E coli* DNA amplified with primers: (A) EAST1; Lane (M) DNA molecular size marker (100-bp ladder); all lanes are positive except lane 12 negative. (B) pet ; Lane(M) DNA molecular size marker (100-bp ladder); lane19negative;remaining lanes are positive.

In the present study, Virulence factors of 86 *E. coli* were studied but we would display data of EAEC strains only. Table (3) summarized the results. Concerning urease enzymes, It was detected in 66/30(20%) of strains in contrast Al-Maliki (2011) found that all isolates of EPEC were negative for urease test [33]. Urease activity in the digestive system contributes to acid tolerance and may promote bacterial survival prior to infection [20].

For hemolysin, lecithinase, Dnase, Congo red binding, all isolates of EAEC was negative. Ali (2006)[24]; Jabuk (2010)[34] and Kadhim et al. (2011)[35] found that all isolates of *E. coli* from stool samples were unable to produce hemolysin .while Al-Chalabi et al. (2010) their results showed that 44(57%) isolates collected as midstream urine samples of uncomplicated UTIs patients were produced hemolysin[36].

The results indicated that 3/30(10%) of EAEC isolates were able to produce capsule also The results indicated that 3/30(10%) of EAEC isolates were able to hydrolysis starch, these results disagree with Ali (2006) [24] and Al-Maliki (2011)[33] who found that 81.8% and 50% of EPEC isolates were capsulated, respectively.

From this research, It was demonstrated that EAEC had high ability for adherence whereas, the results showed the 1/30(3.3%) and 30/30(100%) of EAEC isolates had type I pili and type III pili, respectively. The biofilm formation was 26/30(86.7%) of EAEC isolates produce biofilm with different degree of thickness. Al-Chalabi *et al.* (2010) also detected the high percent (90%) of *E. coli* ability for biofilm formation [36].

The percentage of β-lactamase production by EAEC strains were very high 93.3% and this is very important in antibiotic choose for treatment of children suffering diarrhea caused by EAEC. In compairsion with other studies as Ali(2006) production of β-lactamase was 93.8% [24]; and Kadhim et al. (2011) β-lactamase test was positive for 57(91.9%) *E. coli* isolates from patients and operating theater from Baghdad hospitals [35].

The results in present research indicated that 10/30(33.3%) of EAEC isolates were unable to ferment sorbitol sugar. Khudor *et al.* (2012) who found the frequency of *E. coli* non sorbitol fermenter (NSF) isolates in human stool samples was 46% [37]. This results were higher than the rate reported by Issa (1997) who reported that NSF isolates from inpatient children was 28.57% [38]; Naaham (2004) who showed isolation rate 27.3% [39]. While Shebib (2000) who reported lower NSF isolates rate 5% compared with the rate in the current research [40].

Table 3: Virulence factors produced by EAEC

Virulence factors \ Isolates	EAEC strains (n=30)	
	No.(+)	**%**
Hemolysin	0	0%
Lecithinase	0	0%
Dnase	0	0%
Urease	6	20%
Starch hydrolysis	3	10%
B-lactamase	28	93.3%
Capsule	3	10%
Congo red	0	0%
Biofilm	26	86.7%
CFAI (type I pili)	1	3.3%
CFAIII(type III pili)	30	100%
Sorbitol ferment	20	66.7%

In the present study, susceptibility test of 86 *E. coli* were studied but we would display data of EAEC

strains only. The results of the antimicrobial drug susceptibility tests are shown in Table (3) and are presented in terms of resistance, intermediate resistance and susceptibility. In this study EAEC isolates were resistance (100%) to Penicillin and Ampicillin, High resistance(96.7%) was observed to each of Ceftriaxone, Cefotaxime and Trimethoprim. and Tetracycline (76.7%), resestance percentage of Both Amikacin and Rifampicin were (40%) and (33.3%) respectively.the higher sensitivity percentage reported by Gentamicin(63.3%), Ciprofloxacin(60%) then Nitrofurantoin (56.7%). Several reports mentioned a high resistance rate in E. coli to penicillin, ampicillin and trimethoprim isolated in Tikrit [41], in Baghdad [35], in Najaf [42], in Babylon [43], One explanation for this could be its widespread use in the treatment of diseases associated with Gram-negative bacteria, especially in children under two years of age with acute infectious diarrhea [44]. Also because they are inexpensive and can be obtained easily without a medical prescription, resistance is probably due to indiscriminate antibiotics usage (drug abuse) which could result in plasmid-mediated antibiotic resistance found to be common in *E. coli* [45]. According to this result, the above antibiotics should not be used for treatment diarrhea and other disease caused by E. coli isolate in Salah Alden hospitals. Ahmed *et al.,* (2009) in Tikrit, Iraq found the prevalence of sensitive 60% to gentamicin [41]. But this result disagree with Ali (2006) reported that (98%) EPEC isolates were resistance to Rifampin [24]. In contrast to results of this study, a high prevalence of susceptibility to ciprofloxacin (100%) was reported by Ahmed *et al.,* (2009) in Tikrit [41]. However, if Fluroquinolones drugs are used widely as the first choice of treatment of diarrhea, especially in Iraq, without effective control of usage of antibiotics is not effectively controlled, a rapid emergence of antibiotics resistance most likely will occur. This finding nearly to the result reported by Ahmed *et al*., (2009) in Tikrit, Iraq found the prevalence of resistance was 70% to Tetracycline [41] while Jabuk (2010) and Al-Maliki (2011) who found that 84.2% and 100% of DEC isolates were resistant to Tetracycline respectively [33, 34].With regard to the multi-drug resistance phenomenon, a strain of DEC is considered as a multidrug resistant if it were resistant to at least three antibiotic classes [46]. However, the present study revealed that a high percentage of EAEC isolates (96.7%) were multidrug resistant showing resistance to a minimum of three classes of the antibiotics tested (Table 5). Present data showed that the incidence of resistance to most antibiotics tested for EAEC isolates is high in Salah Al-den.

Table (4) Antimicrobials susceptibility patterns of EAEC

Isolates / Antibiotics	EAEC strains (n=30)		
	S no. (%)	**I no. (%)**	**R no. (%)**
Penicillin	0(0%)	0(0%)	30(100%)
Ampicillin	0(0%)	0(0%)	30(100%)
Ceftriaxone	1(3.3%)	0(0%)	29(96.7%)
Cefotaxime	0(0%)	1(3.3%)	29(96.7%)
Gentamicin	19(63.3%)	5(16.7%)	6(20%)
Amikacin	8(26.7%)	10(33.3%)	12(40%)
Rifampicin	14(46.7%)	6(20%)	10(33.3%)
Ciprofloxacin	18(60%)	5(16.7%)	7(23.3%)
Nitrofurantoin	17(56.7%)	7(23.3%)	6(20%)
Tetracycline	6(20%)	1(3.3%)	23(76.7%)
Trimethoprim	1(3.3%)	0(0%)	29(96.7%)

S: 34ensitive; I:intermediate; R:resestance

Table (5) Antimicrobial resistance phenotype and virulence genes in *E coli*

DEC group	No. Iso.	Antibiotic Resistance No.	Antibiotic Resistance phenotype	Virulence genes EAST1	Virulence genes pet
EAEC	10	5	P;AM;CRO;CTX;TMP	+	+
	25*	4	P;AM;CRO;CTX	—	+
	28	7	P;AM;CRO;CTX;RA;TE;TMP	+	—
	32	7	P;AM;CRO;CTX;RA;TE;TMP	+	+
	39	9	P;AM;CRO;CTX;CN;AK;RA;TE;TMP	+	—
	52	11	P;AM;CRO;CTX;CN;AK;RA;CIP;F;TE;TMP	+	+
	56	6	P;AM;CRO;CTX;AK;TMP	—	+
	58	5	P;AM;CRO;CTX;TMP	—	+
	66	7	P;AM;CRO;CTX;CIP;TE;TMP	—	+
	75	5	P;AM;CRO;CTX;TMP	—	+
	120	6	P;AM;CRO;CTX;TE;TMP	+	+
	134	8	P;AM;CRO;CTX;CN;CIP;TE;TMP	+	+
	151	8	P;AM;CRO;CTX;CIP;F;TE;TMP	+	—
	159	8	P;AM;CRO;CTX;AK;CIP;TE;TMP	—	+
	175	4	P;AM;TE;TMP	—	+
	197	6	P;AM;CRO;CTX;TE;TMP	—	+
	201	7	P;AM;CRO;CTX;CIP;TE;TMP	—	+
	204	7	P;AM;CRO;CTXF;TE;TMP	—	+
	216	8	P;AM;CRO;CTX;CN;AK;TE;TMP	+	—
	220	7	P;AM;CRO;CTX;RA;TE;TMP	—	+
	226	7	P;AM;CRO;CTX;AK;TE;TMP	+	—
	228	6	P;AM;CRO;CTX;RA;TMP	+	+
	229	8	P;AM;CRO;CTX;CN;AK;TE;TMP	+	—
	238	9	P;AM;CRO;CTX;AK;RA;F;TE;TMP	—	+
	241	7	P;AM;CRO;CTX;F;TE;TMP	+	—
	250	9	P;AM;CRO;CTX;AK;RA;CIP;TE;TMP	—	+
	255	8	P;AM;CRO;CTX;AK;CIP;TE;TMP	+	—
	256	9	P;AM;CRO;CTX;AK;RA;F;TE;TMP	+	+
	257	10	P;AM;CRO;CTX;CN;AK;RA;F;TE;TMP	+	—
	260	5	P;AM;CRO;CTX;TMP	—	+

P. Penicillin:AM. Ampicillin:CRO. Ceftriaxon:CTX. Cefotaxime:CN. Gentamicin:AK. Amikacin:RA. Rifampicin:CIP. Ciprofloxacin:F. Nitrofurantoin:TE. Tetracycline:TMP. Trimethoprim

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عزل وتشخيص الاشيريكيا القولونية المتجمعة المعوية ضمن مسببات الاسهال البكتيري في الاطفال

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الملخص

الاسهال مشكلة صحية ومسبب مهم للاصابة والوفاة، الاشيريكيا القولونية المتجمعة ظهرت بشكل متزايد كمسبب للاسهال في الدول النامية والصناعية. فهدف البحث هودراسة انتشار هذه البكتريا وعوامل ضراوتها ومدى مقاومتها للمضادات الحيوية. اضهرت نتائج هذه الدراسة ان نسبة عزل الاشيريكيا القولونية كان 32.9%من مجموع المسببات البكتيرية وان 60%منها كانت من مجموعة الاشيريكيا القولونية المتجمعة المعوية.

البادئ المتخصص pet هو الاكثر انتشارا (28%)من البادئ المتخصص EAST1 (18%) اما نسبة وجود البادئين سوية بنفس السلالة فكانت بنسبة 14% ضمن سلالات الاشيريكيا القولونية المتجمعة المعوية. وجد ان 10% من العزلات تحتوي المحفظة، لكن 3.3% من العزلات تمتلك الاهداب من النمط الاول بينما جميع العزلات حاوية على الاهداب من النمط الثالث. دلت نتائج هذه الدراسة ان كل عزلات الايشيريشيا القولونية المتجمعة المعويةكانت سالبة لاختبار صبغة احمر الكونكو على العكس فان نسبة عالية من العزلات قادرة على تكوين غشاء حيوي 86.7% وبدرجات مختلفة من السمك. ان 20% من العزلات تنتج انزيم اليوريز بعد 24–48 ساعة من الحضن. وان 10% من السلالات تنتج انزيم الاميليز بينما جميع العزلات غير قادرة على انتاج الانزيم الحال للدم وانزيم اللسيثنيز والنيوكليزيينما 93.3% من عزلات الايشيريشيا القولونية المتجمعة المعوية تنتج انزيم بيتا لاكتاميز. نسبة العزلات المخمرة للسوربيتول كانت 66%.في هذه الدراسة كانت عزلات الايشيريشيا القولونية المتجمعة المعوية مقاومة للبنسلين والامبسلين بنسبة 100% وابدت مقاومة عالية 96.7% لكل من الجيل الثالث من السبورينات والتريمثبريم اما تجاه التتراسايكلين فمقاومتها 76.7%اما نسبة المقاومة لكل من اميكاسين وريفامبين فكانت 40%و33.3% بالتعاقب.اما اعلى نسبة حساسية فسجلت للجنتاميسين 63.3% ثم السبروفلوكساسين 60% ثم نايتروفيرانتوين 56.7%. بيانات هذه الدراسة تلفت الانتباه الى اهمية مرض الاسهال، كذلك لوحظ في هذه الدراسة المستوى العالي لمقاومة المضادات الحيوية الذي يثير مناقشة طويلة حول الاستعمال غير المقيد او سوء الاستعمال للمضادات الحيوية واخطار العلاج بالمضادات الحيوية تجريبيا في الاطفال حديثي العمر.