Genetic Survey of Enteroaggregative E.coli in Diarrheic Children under 5 years in Thi-qar governorate

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Abstract

The study aimed to investigate the prevalence of Enteroaggregative E.coli obtained from children of Thi-qar suffered from diarrhea through PCR detection of virulence factors (aggR, astA, pic) antibiotic resistance and extended spectrum β-lactamase (ESBL) production. The investigation included collection 430 stool samples from diarrheic children under five years involved both gender and 40 samples as a control from healthy children , were consulted in (Bint al- Huda Hospital for Gynecology obstetrics and pediatrics , Al-Mousawi hospital and private clinics laboratory) at a period extended from 10th December 2018 -21th July 2019. A total of the isolates were diagnosed by different laboratory and molecular method. PCR diagnosis confirmed the distribution of genes (aggR and astA) within EAEC were (66% and 53%), respectively. The highest resistance to antibiotics was (100%) to ceftazidime and cefpodoxime , (97%) to ceftriaxone, followed by (96%) for Ampicillin, Sulfa-Trimethoprim and cefuroxime, respectively, and cefixime, amoxiclav, Cefotaxim and Tetracycline (94.8%), (92.3%), (85%) and (78.2%), respectively. While the medium resistance was (55.13%) for each of ciprofloxacin , piperacillin-tazobactam, respectively, and Amikacin (51%). While the lowest resistance was to Gentamicin (30.7%) and Imipenem (26.9%). EAEC isolates were multidrug resistance in average 64/66 (96.97%).

Key words: *Enteroaggregative E.coli, virulence genes, antibiotic resistance, ESBL.*

Introduction

Diarrheal diseases are still a global problem around the world especially in children under five years and are among the commonest causes of death among infants and children in developing countries, where poor hygiene and the absence of access to fundamental sanitation enable their dissemination. Worldwide diarrhea takes Order advanced on average death and UNICEF still considers diarrhea as one of the causes of death in young children under 5 years of age, it comes second after death rates of pneumonia [(1)]. Diarrheagenic Escherichia coli (DEC) is a common bacterial cause of acute and persistent infectious diarrhea, play a major role in children under 5 years of age. DECs are classified into six groups based on clinical associations, phenotypic assays and factors of virulence: Enteroaggregative E. coli, Enteropathogenic E. coli, Enterotoxigenic E. coli, Enteroinvasive E. coli , Shiga toxin-producing E. coli, and Diffusily adherence E. coli [(2-3)]. Enteroaggregative E.coli is important one that has been implicated as an emerging cause of traveler's diarrhea and persistent diarrhea among children and immunocompromised patients in both developing and developed countries. Enteroaggregative E.coli characterize by the primary infection mechanism start with attachment to intestinal mucosa by aggregate adherence fimbriae and is controlled by the agg gene , as well as other secreted proteins that play a major part in pathogenicity. Culture and biochemical test can't distinguished between commensal or pathogenic strains of E.coli in stool so PCR used to detect the virulence genes in pathogenic strains [(4)] . PCR provide detection to many Diarrheagenic E.coli strains virulence genes with high sensitivity and specificity [(5)].

Materials and Method

Collection of samples

During the period from 10th December / 2018-21th July/ 2019, a total of 430 stool samples were collected from both gender of diarrheic children under five years had been admitted at hospitals and attended at

private clinics in Thi-Qar province, Iraq. Otherwise, 40 samples of stools from healthy children were obtained as control. The stool samples transported on Carry Blair swabs and cultured on MacConkey agar, Blood agar, XLD and EMB, incubated aerobically at 37 °C for 24 hours, the isolated bacteria were identified according to morphological, biochemical tests, API 20E and VITIK-2 system.

Antibiotic susceptibility test: performed by Kirby-Bauer procedure on Muller Hinton agar and results interpreted according to Clinical and Laboratory Standards Institute. All isolates were tested for ESBL production using the combined-disk test using ceftazidime (30 µg) and cefotaxime (30 µg) disks and combination with clavulanic acid (10 µg) disk and interpreted findings according to the Institute for Clinical and Laboratory Standards [6].

Genetic diagnosis: It preformed by PCR technique that was used for amplifying the virulence genes. The mixture reaction was performed in a total volume 20 µl of PCR Mastermix Gold Monoplex 5x (Mastermix 5 µl , DNA Template 2 µl, Forward primer 1 µl for each primer, Reverse primer 1 µl for each primer, free water ddH_2O 11µl). PCR cycling program parameters used in this reaction for detection of (aggR , astA, pic) genes, the thermal cycling program(Initial denaturation 95°C for 3 min. 1cycle), (Denaturation 94°C for 45 sec 30 cycle), (Annealing 58°C for (aggR ,astA) and 59°C for (pic) 45 sec. 30 cycle), (Extension 72°C for 50 sec.. 30cycle), (Final extension 72° C for 10min. 1 cycle) (Holding 4° C 1 cycle). The amplification products were electrophoresed through a 2 % agarose gel and visualized with UV transilluminator after RedSafe™ staining. A 5 µl of 100 bp DNA ladder was used as a molecular size marker in a gel. Statistical analysis of all variables and comparisons was performed with Excel application (version 2010), Windows 7 and Statistical Package for the Social Sciences (SPSS) (version 22). Chi-square test was used for independent under P-value ≤ 0.05 was considered statistically significant [7].

Primers Sequence (5' – 3')	Product size	References
16SrRNA F: AGAGTTTGATCMTGGCTCAG	1500	8
R: CGGTTACCTTGTTACGACTT		
aggR aggR-F: ACGCAGAGTTGCCTGATAAAG	400	9
aggR-R: AATACAGAATCGTCAGCATCAGC		
astA astA-F: TGCCATCAACACAGTATATCCG	102	9
astA-R: ACGGCTTTGTAGTCCTTCCAT		
pic pic-F: AGCCGTTTCCGCAGAAGCC	1,111	9
pic-R: AAATGTCAGTGAACCGACGATTGG		

Results

The percentage of positive bacterial infections were 180 (41.86%) while the rate of 250 (58.14%) represented other causes of diarrhea E.coli were isolated in 100(23.26%) of 430 collection samples followed by 80(18.60%) represented of other gram-negative bacteria (Salmonella, Klebsiella, Proteus, Pseudomonas, Enterobacter, Serratia, Cronobacter). Enteroaggregative E.coli occurred as the most present strain when reached 66/100 (66.0%), especially in children under two years while it not appeared in healthy children (control patients).

The appearance of aggR, astA and pic virulence genes EAEC isolates were rate 66 (66%), 53 (53%), and 6 (6%), respectively. The fact of aggR gene appearance in all EAEC isolates may be means all of them were

typical EAEC.

The distribution of patients infected with E.coli according to gender which were 53(53.00%) in males and 47(47.00%) in females, while the patients infected with strain Enteroaggregative E.coli were 37(37.00%) in males and 29(29.00%) in females . This results show no statistical significant differences between both gender.

The highest rate of infection exhibited artificial fed with rate 31/100(31.00%) while breast fed and mixed fed reduced to 15/100(15.00%) and 20/100(20.00%), respectively.Show table (1).

Tabal(1):prevalence of the infected children with Enteroaggregative E.coli according to type of feeding .

Infection / Feeding	Enteroaggregative E. coli		Other Strains of E. coli		Total	
	No.	%	No.	%	No.	%
Breast FE	15	15.0	18	18.0	33	33.0
Artificial FE	31	31.0	8	8.00	39	39.0
Mixed FE	20	20.0	8	8.00	28	28.0
Total	66	66.0	34	34.0	100	100
Calx² = 9.737	Tabx² = 5.99		DF= 2		P. Value= 0.008	

The high incidence of EAEC infection was recorded in first and second age group flowed by third and fourth age group, but there were no cases recorded in fifth age group as shown in figure(1).

Antibiotic resistance patterns

The results of antimicrobial susceptibility testing of the 66 EAEC isolates agaist 15 antibiotics are summarized in Table (5). All isolates (100%) were resistant to Ceftazidime and Cefpodoxime, followed by Sulfa-Trimethoprim, Ampicillin and Ceftriaxone 63 (95.5%) then 61 (92.4%) for Amoxiclav and Cefixime, 56 (84.9%) Cefotaxime,36 (54.6%) Ciprofloxacin, Piperacillin –tazobactam and Amikacin 34 (51.5%). While the lowest resistance was to Gentamycin and Imipenem that was 20(30.3%) and 18 (27.3%) , respectively.

ESBLs Phenotype

The overall occurrence of ESBL-producing EAEC isolates was 56.06% (37/66). All isolates that were tested positive for ESBLs were also MDR.

Discussion

In present study, the most common type of Diarrheagenic E.coli was EAEC, which may be largely responsible for diarrhea in children.

The most common type of diarrheagenic E.coli was EAEC, which was prospect largely responsible for diarrhea in children with prevalence 66%,this result near with globally studies such as by Abimiku et al [8] in Nigeria (68.67%) ,Mandal et al[10] in India (69%). The out result more than local and globally studies such as Abdul-Hussein et al [5] in Kut who showed incidence of EAEC was (40.5%) and Zhou et al[11] in Chin reported (20%), that reported a high a frequency of EPEC pathotypes associated with pediatric diarrhea.

Three predominant genes detected in EAEC from diarrheic children in Thi-qar were aggR (66%), astA

(53%), and pic (6.0%).This results corresponding with Ali et al[12], they were reported relatively similar findings for aggR (90%), astA (52.6%) and pic (5.3 %) .

The present study with PCR showed no-statistical significant according to gender. The current study agreed with Ammary and Pediatrics [13] in Baghdad ,Aslani et al[14] in Iran, when was detect differences between males and females infection rate, males was higher than females. The virtual but not statistical high rate of infection in males may be due to males are more active, and more mixing in society Thi-qar than females.

Molecular analysis proved statistical significant according to type of feeding where the infection rate in artificial fed more than mixed fed and breast fed. These results corresponded with some previous studies as the study by Ali et al [12] in Egypt and Ammary and Pediatrics [13] in Baghdad .The high incidence of infection in artificial fed and mixed fed may be due to several factors including; poor hygiene, sanitation, Contamination , the widespread spread of bacteria, particularly in low-birth communities social and economic [14].

Habitation did not excite statistical significant in infection rate that was (56.06%) in rural while decreased in urban (43.94%). These results consistent with some previous studies Gutiérrez-Jiménez et al[15] in Mexico whose noticed infection rate of EAEC in rural (52%) compared with urban (48%), also the current results were comparable to the results of Ammary and Pediatrics [13] in Baghdad, were said (64%) positive samples from rural and (36%) positive samples in urban.

Enteroaggregative E. coli pathotype were detected in each one of 4 age group , but the most cases infection were occurred among children in first and second years , this result consistent with local study reported by Khalil [2] in Baghdad showed high prevalence of EAEC in children less than 2 years. Some researches close agreement with this results involved Nguyen et al [16]) in Vietnam who showed that EAEC was more frequently isolated in children less than 2 years .

Antimicrobial Susceptibility Pattern

Our study for Cefpodoxime and Cefuroxime was agreement with global studies by, Amin et al [3] 81.3%, Karami et al [17] 97 % and Rodríguez-Baño et al [18] 97.3. Resistance to Ceftriaxone and Cefotaxime (100,85%),respectively agreement with another study had been reported high resistance in studies done by Sakhi [19] 85.7% and AL-Hilali [20] 68%.

Resistance to Amoxiclave (92.32%), was agreement with another studies as done by Al-Hilali [20] in Al-Najaf who motioned resistance 83.4 % and also with the study of Khoshvaght et al [21] in Iran with rate 86.11% . Resistance to ampicillin and sulfa-Trimethoprim (96.1%), were agrees with Aslani et al [14] and Amin et al [3], hose reported 100% resistance for each one

Similar study by Sakhi [19] in Thi-qar indicated highest resistance in E.coli isolates to Tetracycline 78%. Another studies involved Konate et al [22] in Burkina Faso whose revealed that 85 % of E. coli isolates were resistance to tetracycline. Therefore tetracycline should not be used without first performing culture and sensitivity [23].

Piperacillin-tazobactam showed resistance similar with global study by Konaté et al [22] whose reported resistance (64.5%). locally Shamki [24] where pointed resistance in rate (60%).

ciprofloxacin showed medium resistance corresponding with globally study by Cassier et al [25] and Khoshvaght et al [21] whose marked out resistance about (61%) and (47%), respectively.

Amikacin, this result corresponds a study performed by Shamkhi [24] . Globally in lined with Zhou et al [11] found that vast majority of E. coli were susceptible to Amikacin, in 7.4%

Gentamycin showed low resistance agreemented with 34% reported Cassier et al [25] and Khoshvaght et al [21] 41%.

Imipenem belong to carbapenem antibiotic binds to different penicillin binding protein. It is from the most active drug against gram negative bacteria , the sensitivity of Enteroaggregative E.coli to imipenem was (44.87%).

The current study found all Enteroaggregative E.coli isolates were multidrug resistance in rate 96.97%. This results agreed with Dembélé [23] in Burkina Faso and each study of Khoshvaght et al [21] and Aslani et al

[14] in Iran.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science and all experiments were carried out in accordance with approved guidelines.

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