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Molecular study of some Virulence Factors Enteropathogenic *Escherichia coli* isolated from newborn till the age of one year

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Abstract
Collected 240 samples from Abu_Ghraib Teaching Hospital, included 120 samples of Stool, 120 samples of urine, for a period from January 07, 2017 to April 07, 2017.The strains of *E. coli* samples were examined in the central laboratories of health in Baghdad, the examination was conducted using polyspecific test reagents and mono specific test reagents. The results polyspecific test reagents show that the dominated serotype is Enteropathogenic *E. coli for ages 1 day to 1 year*. The results of mono specific test reagents show that the percentages of serotype are: O103 (25,7%) overall and; 60% in stool and 40% in urin, O26 (20.57%) (61%S,39%U), O78(18.85%) (54.5%S,45.5%U), O25 (12.57%) (50%S,50%U), O118 (10.85%)(53%S,47%U), O142 (8.57%) (47%S,53%U). The resistance results of the bacterial sensitivity tests for 15 antibiotics on the *E. coil* were: Penicillin G (100%), Erythromycin (99.5%), Tetracycline (99.5%), Cephalothin (99.4%), Clindamycin (99%), Trimethoprim (98%), Amoxicillin (90.9%), Cefotaxime (89.7%), Chlorphenicol (79.4%), Ampicillin (63.4%), Netimicine (38.9%), Nalidixc Acid (33.7%), Norfloxacine (12%), Ciprofloxacine (10.9%), Tigecycline (0%). 30 isolates were selected for *E. coli* strains which included 20 stool isolates and 10 urine isolates to examine the presence of 6 virulence genes using the polymerase chain reaction technique (PCR), The percentages of virulence genes were: eaeA gene (63.3%), Stx_1 gene (100%), UidA gene (96.66%), neuC gene (23.3%) and hlyA gene (80%). The bfp gene was not obtained in the study samples because it is a diagnostic gene.

INTRODUCTION

The *Escherichia coli* is an endemic bacterium in the digestive tract of children After a few days of birth, which compose the Normal flora, it was isolated from the healthy children's stools so it is considered as Non-pathogenic [1]. *E. coli* has many Virulence Factors that help it to cause many diseases, so it is divided into several types according to the disease occurrence location [2]. The *E. coli* that cause inflammation of the bowel, can be cause about 30-40% of diarrhea that affects infants [3]. Enteropathogenic E. coli (EPEC) pattern which composing Attaching and Effacing lesions (A/E), Which stick onto the intestinal epithelium and causes diarrhea of varying severity for infants with mucus and without blood. Enterohaemorrhagic *E. coli* (EHEC) pattern or Known as bacteria Vero-toxin is a producer of (Shiga-toxin) which causing a bloody diarrhea for children and the O157:H7 is the most common strain of this pattern. Enterotoxigenic *E. coli* (ETEC) pattern stick onto the surface of the intestinal cells, excrete toxin and cause watery diarrhea associated with some of the symptoms such as gastric colic abdominal, acidosis, fatigue, dryness and high temperature, and this because of the possession of some factors of ferocity LT, ST enterotoxin. Enteroinvasive *E. coli* (EIEC) pattern which invade the mucous layer and subcutaneous mucosa, and also causing diarrhea which called Dysentery diarrhea with blood and mucus. Enrtoaggregative *E. coli* (EAEC) pattern stich onto the intestinal cells and form a thin layer of mucus on them, causing chronic diarrhea which continue for more than 14 days in Children. Adherence *E. coli* (ADEC) pattern which is the last group that discovered and causes diarrhea for Children, its name derived from its prescription which is adhering to Fimbria-mediated [4,5,6]. *E coli* can cause infections in other locations such as urinary tract infection, meningitis and Bile duct inflammation.

MATERIAL AND METHOD

Collected about 240 samples from newborn for ages one day up to one year at Abu-Ghraib teaching hospital, they included 120 stool's samples and 120 urine's samples. The tested samples were sent to the central health laboratory in Baghdad to diagnose the serotypes using the diagnosis that supplied from Sifin company. And then testing the sensitivity of the *E. coli* to 15 antibiotics using the Kirby Bauer method. The bacterial DNA was then extracted according to extraction Kit supplier procedure (Gene aid company), The polymerase chain reaction technique was then conducted to investigate some of the virulence genes using the kit of pioneer company.

RESULTS AND DISCUSSION

Diagnosis of Serotypes

The serological patterns of *E. coli* carried by children isolates were identified and the results show that the most common serotype in children was Enteropathogenic *E. coli* (EPEC), and no other patterns were obtained, This result was similar to what was found in some studies, this pattern was found in Basra (52%) [12], in Baghdad (29.4%) [13], in Iran (56.7%) [14], in Australia (14%) [15], in Nigeria (16%) [16], in Europe - lima (7.6%) [17]. These percentages varied between provinces and countries may be due to different environmental conditions, different ages of children, the social situation and the length of time that this research was conducted in. The serological strains obtained are:

Sensitivity Test

The sensitivity of *E. coli* was tested to 15 Antibiotics by Kirby & Bauer Method and the results was consistent to those obtained in similar researches conducted by other researchers such as [18, 19]:

Table of Primers

Gene	Primer 5-----3	Product size (bp)	Refernces
neuC	F:AGGTGAAAAGCCTGGTAGTGTG R:GGTGGTACATCCCGGGATGTC	676	[7]
uidA	F:GCGTCTGTTGACTGGCAGGTGGTGG R:GTTGCCCGCTTCGAAACCAATGCCT	510	[8]
hlyA	F:TGGTGCAGCAGAAAAAGTTG R:CCCGTTGTTTTCTCAGCAAT	233	
Stx_1	F:AAATCGCCATTCGTTGACTACTTCT R:TGCCATTCTGGCAACTCGCGATGCA	366	[9]
eaeA	F:TGAGCGGCTGGCATGAGTCATAC R:TCGATCCCCATCGTCACCAGAGG	241	[10]
bfp	F:AATGGTGCTTGCGCTTGCTGC R:GCCGCTTTATCCAACCTGGTA	324	[11]

Table of program of the polymerase chain reaction system for the reaction mixture

	Initial denaturation	Denaturation	Primer annealing	primer extension	Final extension	Refernces
neuC	°95c{5min*1}	95c{30sec*30}	58{30sec*30}	72c{30sec*30}	72c{5min*1}	[7]
UidA	°95c{5min*1}	95c{30sec*30}	65{30sec*30}	72c{30sec*30}	72c{5min*1}	[8]
hlyA	°95c{5min*1}	95c{30sec*30}	60{30sec*30}	72c{30sec*30}	72c{5min*1}	
Stx_1	°95c{5min*1}	95c{30sec*30}	65{30sec*30}	72c{30sec*30}	72c{5min*1}	[9]
eaeA	°95c{5min*1}	95c{30sec*30}	68{30sec*30}	72c{30sec*30}	72c{5min*1}	[10]
bfp	o95c{5min*1}	95c{30sec*30}	61{30sec*30}	72c{30sec*30}	72c{5min*1}	[11]

Table of Serological Strains

serotype	Female/stool%	Female/urine%	Male/ stool%	Male/ urine%	
O103	51.1	28.9	8.9	11.1	%25.7
O78	27.9	19.4	33.3	19.4	%18.8
O26	39.3	30.3	15.2	15.2	%20.5
O25	18.2	9.1	31.8	40.9	%12.5
O118	21.1	26.3	31.5	21.1	%10.8
O142	26.7	33.3	20	20	%8.5
Normal	40	0	60	0	%2.8

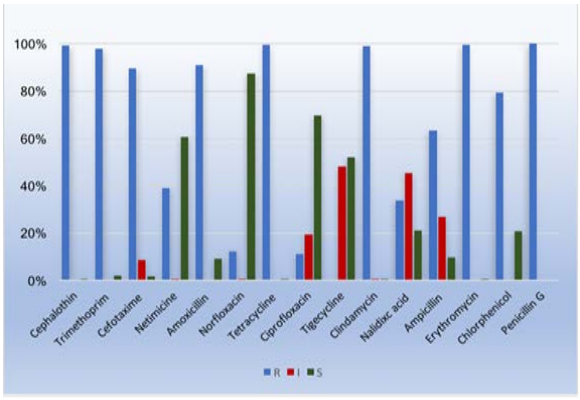

Figure for results Antibiotic

The cause of bacterial resistance to these antibiotics May be because of the change of Membrane barrier permeability which making the passing of antibody difficult to reach the target's site or as a result of the secretion of Betalactam enzymes [20].

Detection of Virulent Genes

Six Virulence genes eaeA, bfp, stx-1, neuC, uidA, HlyA, which may be carried by *E. coli* and encode some of the Virulence factors that help to induce diseases, were found by polymerase chain reaction technology. 30 samples were selected based on the resistance to the antibiotic and the presence of virulence factors, including 20 stool samples and 10 urine samples.

The **eaeA** gene, which encodes the Intimine protein in *E. coli*, was found to be 63.3% at size 241bp. it was observed with high rate in O78 strain and normal flora *E. coli which were* about (100%) in both, in the other strains as follow: O103 by (66.7%), O118 by (50%), O142 by (25%) and there were no positive results for this gene in strain O25. These results partially correspond with the results of [9,10, 21].

The bfp gene, which plays a role in the adhesion of *E. coli* to the intestinal wall by Bundle Forming Pilus (bfp), is a diagnostic gene for the typical *E. coli*, showing negative results in the presence of this gene at volume (324 bp). These results is consistent with those reached by [11,21].

The Stx-1 gene, which encodes shiga-toxin that producted by the bacteria, has been detected as a virulence factors of the diseases. The results show that this gene was 100% at molecular size (bp366). All serotypes O103, O26, O78, O25, O118, O142 and Normal flora show 100% in the presence of this gene. This finding correspond with the finding of [9, 22].

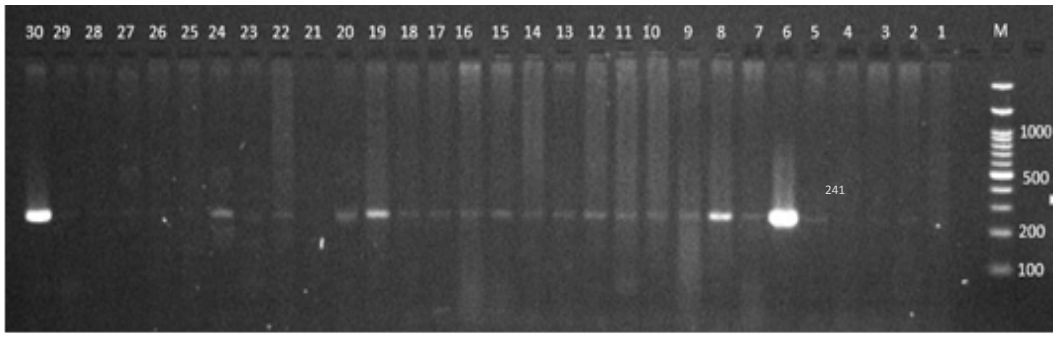

Figure of the electrical displacement to investigate the presence of the eaeA gene at molecular size bp241 represents the M volumetric index bp100 as the samples are represented O118 (25 ,22 ,12 ,1), O26 (27 ,24 ,13 ,5 ,2), O25 (23 ,3), O142 (26 ,21 ,6 ,4), O78 (18 ,11 ,11 ,8 ,7), Normal flora (20 ,19 ,16 ,10 ,9), O103 **(**30 ,29 ,28 ,17 **,**15 **,**14)

Figure of the electrical displacement to investigate the presence of the bfp gene at molecular size bp324 represents the M volumetric index bp100 as the samples are represented O118 (25 ,22 ,12 ,1), O26 (27 ,24 ,13 ,5 ,2), O25 (23 ,3), O142 (26 ,21 ,6 ,4), O78 ,11 ,8 ,7) (18 ,11, Normal flora (20 ,19 ,16 ,10 ,9), O103(30 ,29 ,28 ,17 ,15 ,14)

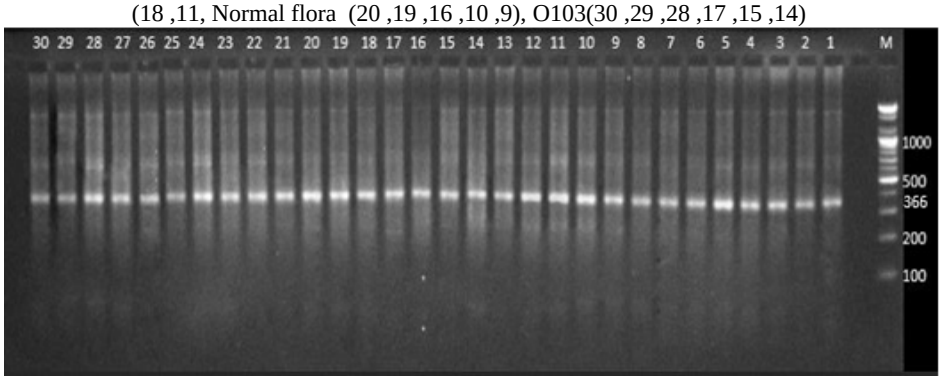

Figure of the electrical displacement to investigate the presence of the Stx-1 gene at molecular size bp366 represents the M volumetric index bp100 as the samples are represented O118 (25 ,22 ,12 ,1), O26 (27 ,24 ,13 ,5 ,2), O25 (23 ,3), O142 (26 ,21 ,6 ,4), O78 ,11 ,8 ,7) (18 ,11, Normal flora (20 ,19 ,16 ,10 ,9), O103(30 ,29 ,28 ,17 ,15 ,14)

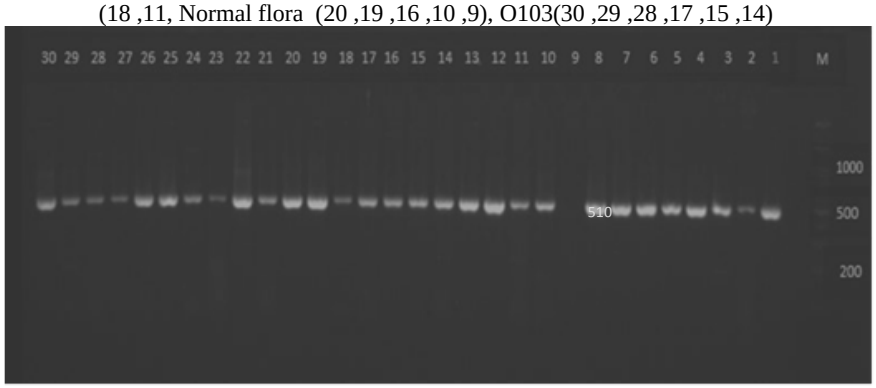

Figure of the electrical displacement to investigate the presence of the uidA gene at molecular size bp510 represents the M volumetric index bp100 as the samples are represented O118 (25 ,22 ,12 ,1), O26 (27 ,24 ,13 ,5 ,2), O25 (23 ,3), O142 (26 ,21 ,6 ,4), O78 ,11 ,8 ,7) (18 ,11, Normal flora (20 ,19 ,16 ,10 ,9), O103(30 ,29 ,28 ,17 ,15 ,14)

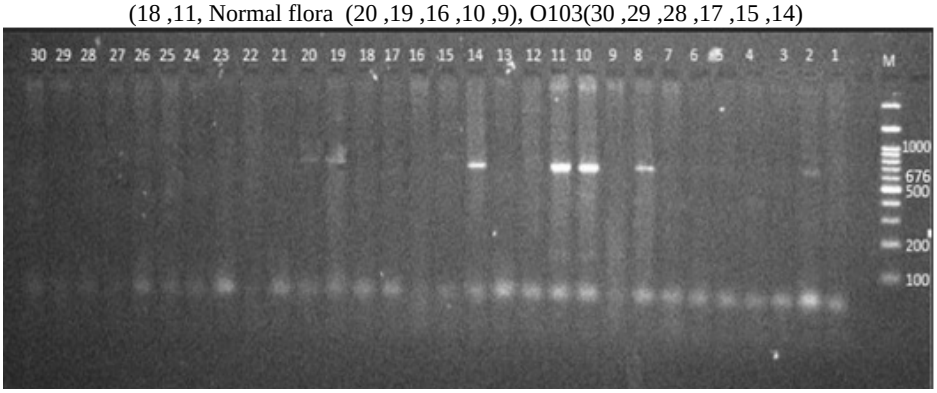

Figure of the electrical displacement to investigate the presence of the neuC gene at molecular size bp676 represents the M volumetric index bp100 as the samples are represented O118 (25 ,22 ,12 ,1), O26 (27 ,24 ,13 ,5 ,2), O25 (23 ,3), O142 (26 ,21 ,6 ,4), O78 ,11 ,8 ,7) (18 ,11, Normal flora (20 ,19 ,16 ,10 ,9), O103(30 ,29 ,28 ,17 ,15 ,14)

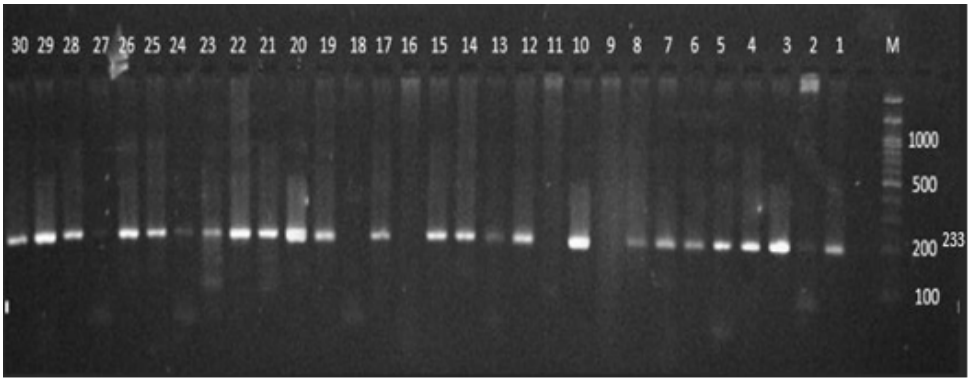

Figure of the electrical displacement to investigate the presence of the hlyA gene at molecular size bp233 represents the M volumetric index bp100 as the samples are represented O118 (25 ,22 ,12 ,1), O26 (27 ,24 ,13 ,5 ,2), O25 (23 ,3), O142 (26 ,21 ,6 ,4), O78 ,11 ,8 ,7) (18 ,11, Normal flora (20 ,19 ,16 ,10 ,9), O103(30 ,29 ,28 ,17 ,15 ,14)

The uidA gene encodes an enzyme Beta _ glucuronid that works on the analysis of sugars. The presence of this gene has been investigated in E. coli bacteria. The results show that it was 96.66% at the molecular size bp510. All strains show 100% presence but normal flora has been found to be 80%. These results are consistent with the findings of [8, 23].

The presence of the neuC gene, which encodes multiple polysaccharides in the capsule (KI Capsule), was detected in *E. coli*. This gene was found by 23% at the molecular size bp 676. The results of the presence in the strains show that normal flora had the highest rate by 60%, and the other rate as follow: O78 by 50%, O26 by 20% and O103 by 16.7%, the remaining strains show negative results of the presence of this gene. This findings are consistent with the results of [7, 24].

The hlyA gene, which encodes the Enteroheamolysin toxins, was investigated, the results show that this gene was 80% at the molecular size bp 233. The results of its presence in strains were 100% in O103, O25, O118, O142 and O26, where in Normal flora and O78 were 75% and 50% respectively, this findings are consistent with the results of [25].

The differences in the presence of genes in the serological strains of *E. coli* in this study with the other studies may be because of the different geographical location from which the samples were isolated, the age of the children, the genus of the children and the economic situation that may affect.

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