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MOLECULAR STUDY OF ENTEROPATHOGENIC *ESCHERICHIA COLI* ISOLATION FROM CLINICAL SAMPLES

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ABSTRACT

Escherichia coli are regarded as the most important causes of intestinal illnesses in the world and also a significant public health challenge. Intestinal sicknesses which are caused by *E. coli* infection are often under diagnosed while doing usual microbiological analysis, particularly in resource constrained settings. However, the use of molecular tests could be helpful to identify the distribution of intestinal illnesses of *E. coli* infection and also its clinical importance. The distribution analysis of virulence factors which exist among various *E. coli* pathotypes which include Shiga toxin-producing *E. coli* (STEC) will offer substantial insights with regard to the mechanisms through which various *E. coli* strains lead to diseases and result in the growth of different *E. coli* types. This present investigation therefore is an attempt to evaluate the incidence of intestinal illnesses of *E. coli* in clinical samples taken from patients. The samples from 150 patients and 15 controls were collected and then screened for *E. coli*. Compatible colonies taken from 150 individuals (150 patients and 15 controls) were characterized through performing biochemical test, a set of Polymerase Chain Reaction (PCR) for recognition of six virulence factors. The clinical samples from patients were within two months -two year attending. Pyelonephritis were associated by pili (*pap gene*, hemolysin (*hly gene*), aerobactin (*aer gene*), translocon pore-forming subunit *espB* and Shiga toxins (Stx1 and Stx2) respectively. They were related with six intestinal illnesses pathotypes. The incidence of *hlyA* and *papC* (both 40%) was highest, and was followed *by espB, aer, stx1, stx2*(30%,25%,20% and 18%) respectively. Although, how and whether these mixtures of genes affect their pathogenicity is an issue that have to be investigated and elucidated. The findings obtained in this study highlight the significance of epidemiological and microbiological surveillance of STECs. It also determines if the development of control measures will diminish the risks which are associated with intestinal illnesses from *E.coli.*

Keywords: molecular, characterises, Enteropathogenic, *Escherichia coli* isolation

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INTRODUCTION

Escherichia coli is considered as an ever present microbial kind commensal of individuals and also animals of warm blooded types. However, some strains are recognized to have developed the ability that leads to both intestinal and extra intestinal sicknesses [1]. *E.coli* bacteria are known as Gram-negative facultative anaerobic bacilli. They are not glomerular and have pertrisial eyelashes for mobility. These bacteria are considered potential causative agents of various diseases such as cholecystitis, pneumonia, pyelonephritis, cystitis, urinary tract infections, neonatal meningitis, septicemia, infections of the central nervous system and

respiratory tract [2].On the other hand, it is worth noting that many *E. coli* strains are commensal flora found in the gut, and the barrier between E. *coli* symbiosis and virulence mainly is dependent on the equilibrium which exists between host immune status and bacterial acquisition capacity viral factor. Infectious diarrheal and gastro has been a main source of disease and death in younger children and is mostly found in developing countries [3]. Enteropathogenic *E. coli* from the Study and Malnutrition and Malnutrition and Malnutrition and Enteric Diseases Encyclopedia identifies the main reasons of moderate to very severe diarrhea in younger children [4].The infection causes severe watery diarrhea with fever, vomiting and dehydration [5].A full profile of virulence factors is the code of all genes. The pathogen *E. coli* with an interpersonal disease is a serious cause of diarrheal, often with a high degree of morbidity, which is reported in developing countries [6]. Among the diarrheagenicpatho types of *E. coli*, and Enteropathogenic *E. coli* are more frequent and frequently occurring in waterborne occurrences worldwide [7].

In particular, in certain geographical markets, for example in some certain developing countries, infections caused by *E. coli* diarrheal have been transferred through human contacts, like enteroaggregative *E. coli* (EAggEC), endemic [8].Furthermore, the treatment with human manures is frequentlyunusable or levellacking. Therefore, it principals to a wide spreading of these pathogens in the surrounding environment and then they might have contact with Shiga toxin-producing *E. coli* (STEC).We examined the presence of pathogenic *E. coli*, mainly entropic ones, in specimens isolated from infant clinical specimens[9].Molecular characterization has manifested high degree of diversity among virulence factors in different strains of atypical EPEC [10].The pathogenicity of degree of extraintestinal *E. coli* isolates is dependent on the existence of fimbriae adhesions *(fimA, sfa/foc, and yfcV)* which are documented as very important factors that are responsible for the related adherence [11]. Adherence is considered as an important requirement for initiating and colonization to convinced host cells Some other actual issues are known as haemolysins (*hylA* and *hylF*) [12].

The purposes of this study were to detect *E. coli* virulence factors in clinical samples collected from hospital from Karbala, Iraq, and to introduce PCR assays as an approach for detection.

MATERIALS AND METHODS

Sample collection

This present study aims at evaluating the number of *E. coli* pathotypes which circulate in younger children with diarrheal in Karbala. Accordingly, stool samples were taken from children (2 month-2 years) of age with diarrheal. A total of (n=150) stool samples and 15 samples control, were collected and processed during. from May 2021 until November 2021. All collected samples were then directly transferred to the laboratory and were analysed within 24 hours.

Sample processing

Escherichia coli isolates were obtained by culturing stool samples were inoculated on eosin methylene blue agar and incubated at 37°C for 24h. identified *E. coli* by biochemical tests of indole production, sugar ferment, Voges-Proskauer reactions, and by the hydrogen sulphide Public Health England [13].

DNA Preparation

The collected cultures of bacterial *E. coli* were Incubated at 37 ^{0}C on nutrient broth for 18 hours. An estimated concentration of each *E. coli* cultures after the incubation period was approximately 10^{6} CFU ml $^{-1}$ of vegetative forms. A bacteriological loop was used to carry part of the one colony into 500 µL distilled water, which was then 30 sec vortexed, suspending culture then, boiled for 10 min and finally centrifuged for 7 min at 14,000 rpm, put on icefor 10 min and centrifuged for 10 min at 14,000 and the supernatant was utilized for PCR [14].

Oligonucleotide primers and PCR conditions

The extracted DNA was, the PCR primers were selected for each enterotoxins which partially amplified the (*stx1, stx2, espB,pap, sfa, hlyA* and *aer* genes each as indicated [15], the positive control (*E.coli ATCC 25992*) showed in (Table 1,2 and 3).

Table 1: Reaction mixture of PCR for detection *E. coli* toxin genes

Component	Volume (µL)
GoTaq Green Master (25)	25.0
Upstream primer (10 µM)	2.5
Downstream primer (10 µM)	2.5
DNA Template	2.5
Nuclease-free water	17.5
Total	50.0

Table 2: primers used for detection of *E. coli* toxin genes

Primer Identified Genes	primer sequences (5'-3')	Product Gene size bp
stx1	F GAAGAGTCCGTGGGATTACG R AGCGATGCAGCTATTAATAA	302 bp
Stx2	F GGGTACTGTGTGCCTGTTACTGG R GCTCTGGATGCATCTCTGGT	510 bp
espB	F GGC GTT TTT GAG AGC CA R GAT GCC TCC TCT GCG A	260 bp
papC	F GACGGCTGTACTGCAGGGTGTGGCG R ATATCCTTTCTGCAGGGATGCAATA	328 bp
aer	F TACCGGATTGTCATATGCAGACCGT R AATATCTTCCTCCAGTCCGGAGAAG	602 bp
hlyA	F AACAAGGATAAGCACTGTTCTGGCT R ACCATATAAGCGGTCATTCCCGTCA	1177 bp

Table 3: Primers used for PCR amplification of detection of *E. coli* toxin genes

Target gene	**Amplification (35 cycles)**				
	Primary denaturation	**Secondary denaturation**	**Annealing**	**Extension**	**Final Extension**
stx1	95 ◦C	95◦C	60◦C	72◦C	72◦C
Stx2	95◦C	94◦C	56◦C	72◦C	72◦C
espB	94◦C	95◦C	60◦C	72◦C	72◦C

papC	95◦C	95◦C	54◦C	72◦C	72◦C
aer	94◦C	95◦C	55◦C	72◦C	72◦C
hlyA	95◦C	94◦C	56◦C	72◦C	72◦C

Agarose gel Electrophoresis

Extracted genomic DNA Agarose gels (1% w/v) were managed in 1xTAE buffer and 1 µL of each DNA extract was electrophoresed at 95 V minutes following PCR amplification.in 1xTAE buffer and post-stained in 1x TAE buffer while GeneRulerTM 1 kb DNA ladder containing 0.1% The gel was pre-stained with MaestrosafeTM Nucleic Acid were utilized as DNA size marker [16]. At last, all related gels were analyzed and captured by UV trans-illuminator Gel Documentation System (Syngene, UK).

RESULTS AND DISCUSSION

All 65 *E. coli O157:H7* isolates were detected to be appeared as gram negative rod under light microscope, mostly motile indole and methyl red tests and negative reactions on voges – proskaure, oxidase and citrate tests. provided positive reactions on catalase, Triple Sugar Iron agar medium provided yellow colour with capability for gas production. The results obtained in this study were in contrast as reported by [17], As they reported *E. coli* Ten bacterial isolates were obtained from n=25 urine samples collected from medical city hospital in Baghdad. These isolates of *E. coli* were identified by cultural and biochemical tests.

The obtained results demonstrated to determine the prevalence of selected virulence genes among *E.coli*, which showed to positive towards *stx1,stx2,espB,PapC,aer,hylA*(6.89%,5.51%,25.51%,31.03%,12.41%&27.58%) would produce amplicons of 302bp,510bp,260 bp,328 bp,602 bp &1177 bp respectively in size (Figure 1-6). The results in our study were in line with [18] Among the adhesions, the Haemolysin A and P fimbriae were the most predominant. The result has been in contrast with A total of 63 clinical samples were collected and screened for the presence of *E. coli O157:H7*. Another study by [19] recognised 239 Shiga toxin-producing *E. coli* strains isolated was observed in 199 separate (83.26%). Haemolytic uremic syndrome patients were ehxA-positive53 (26.63%) and 99 (49.75%) faces (diarrheal circulation). The results obtained in this study were in contrast as reported by [20] *stx1* profile was the furthermost public and was obtainable in 20.4% (202/990) of the isolates, followed by *stx2* (4.54%; 45/990) and *stx1*and *stx2* profile (2.92%; 29/990). The findings were compatible with what mentioned showing that such characteristics usually are coming in accordance with those which belong to *E. coli* [21].

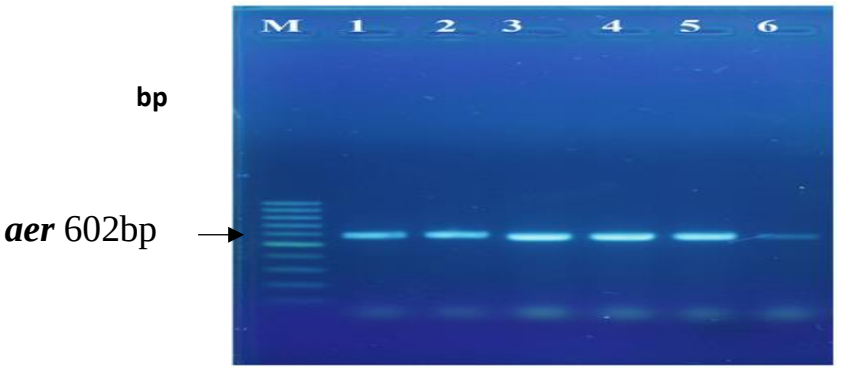

Figure 1: Agarose gel electrophoresis of PCR product of *aer* gene (602 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-6 *E. coli* isolates. Lane 1: (*E.coli ATCC 25992*) positive control.

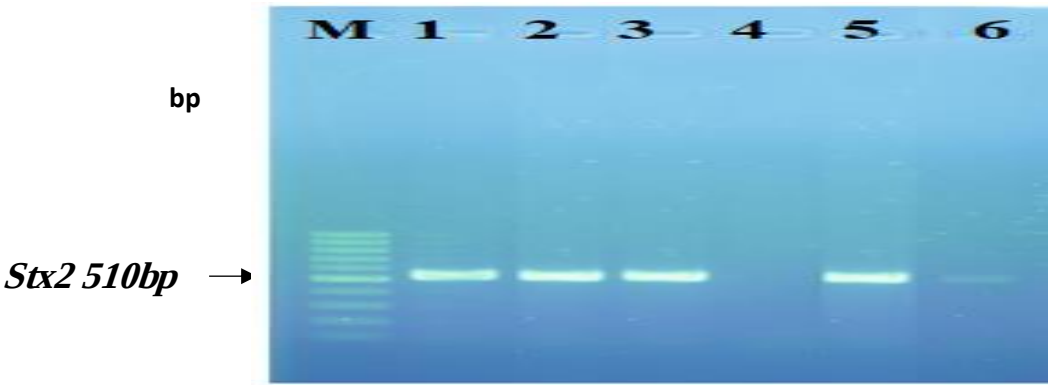

Figure 2: Agarose gel electrophoresis of PCR product of *Stx2* gene (510 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-6 *E. coli* isolates. Lane 1: (*E.coli ATCC 25992*) positive control.

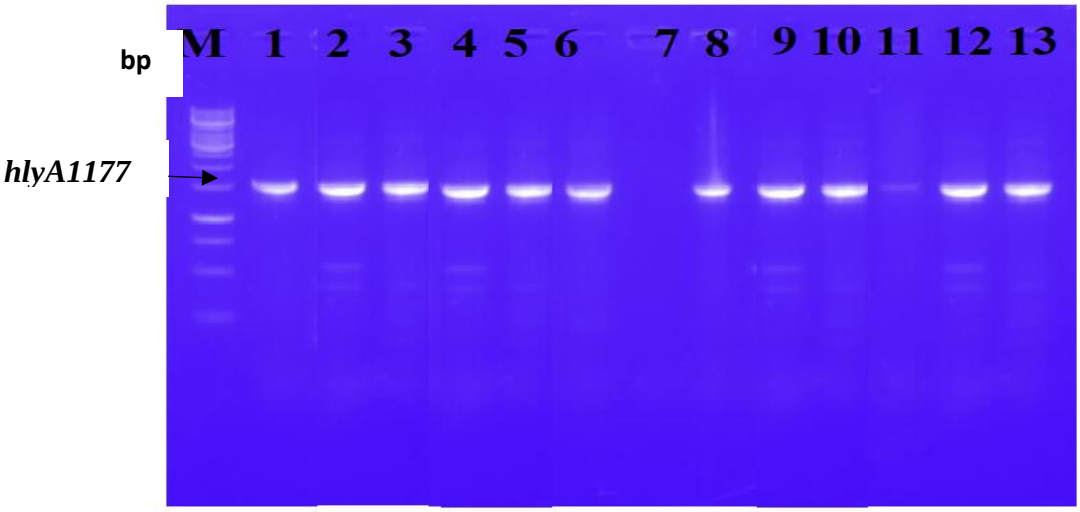

Figure 3: Agarose gel electrophoresis of PCR product of *hlyA* gene (1177 bp) amplified from some of *E. coli* isolates, electrophoresed on 1 % (w/v) agarose gel. Lane M: 10000 bp DNA ladder; lanes: 2-13 *E. coli* isolates. Lane 1: (*E.coli ATCC 25992*)positive control.

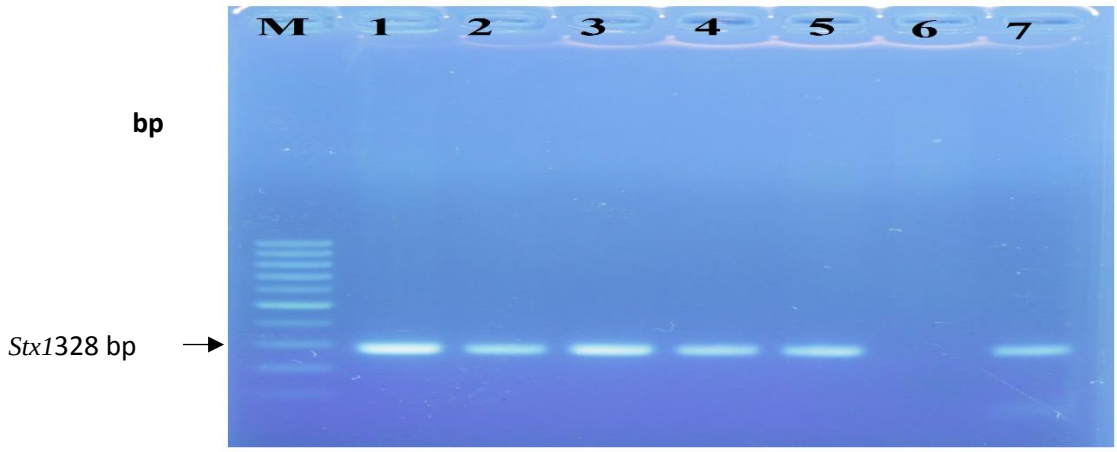

Figure 4: Agarose gel electrophoresis of PCR product of *Stx1* gene (328 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-7 *E. coli* isolates. Lane 1: (*E.coli ATCC 25992*) positive control.

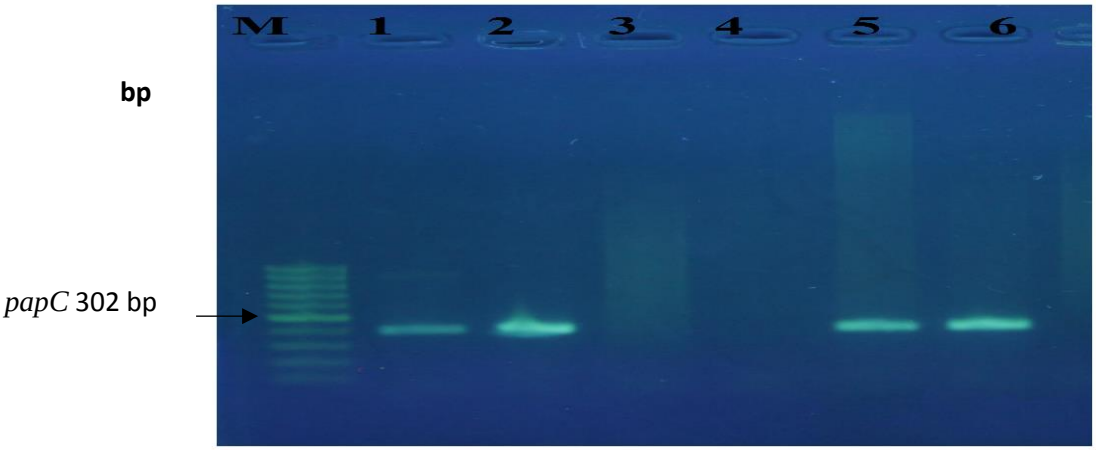

Figure 5: Agarose gel electrophoresis of PCR product of *papC* gene (302 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-6*E. coli* isolates. Lane 1: (*E.coli ATCC 25992*) positive control.

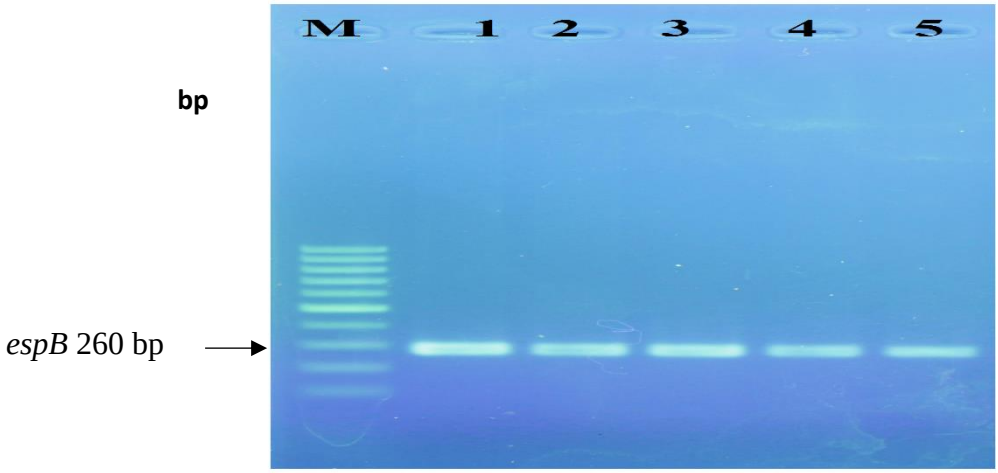

Figure 6: Agarose gel electrophoresis of PCR product of *espB* gene (260 bp) amplified from some of *E. coli* isolates, electrophoresed on 1% (w/v) agarose gel. Lane M: 100 bp DNA ladder; lanes: 2-5 *E. coli* isolates. Lane 1: (*E.coli ATCC 25992*) positive control.

CONCLUSION

There is a high incidence of *E. coli* in human and their environment. The statistics explained in the current study specifies a very great expression of the diarrheal toxin genes of *E. coli* isolated in medical samples demonstrating that the pathogenic strains of the microorganism are highly adapted for toxins.

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