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RESEARCH ARTICLE

Molecular Detection of *hlyA* Gene from *Escherichia coli* hemolytic isolated from Intestinal and Urinary tract infections

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Abstract

An Intestinal and Urinary tract infection is a common clinical problem worldwide. In this study 76 isolates *Escherichia coli* were isolated from midstream urine specimens only 30 isolates show ability to hemolysis, and 80 isolated *Escherichia coli from* diarrhea specimens only 10 isolates show ability to hemolysis. In this study, *hlyA* gene responsible for hemolysin production were detected, from fifteen clinical isolate distributed on 10 isolates of urine and 5 isolates of diarrhea of *E.coli* collected from 40 isolates for hemolysin produced by using PCR techniques. *hlyA* gene was present in all isolates.

Keywords: *Hlya, Escherichia coli, Hemolysin.*

Introduction

Escherichia coli is one of the most important intestinal family species and its natural habitat in the human digestive tract, which makes it able to injure the body opportunistically when there is an opportunity to do so and cause many diseases [1] *E. coli* is the most common cause of intestinal and extra-intestinal disease [2]. Intestinal or extra-intestinal *E. coli* infections are caused by strains harboring numerous virulence factors located on plasmids, bacteriophages, or the bacterial chromosomes. Several studies have shown that pathogenic *E. coli* strain may be derived from commensal strains by the acquisition of chromosomal or extra-chromosomal virulence operons [3].

Escherichia coli is one of the most important pathogenic bacteria that share the events of microbial contamination and cause about 90% of the urinary tract infection [4]. Diarrhea is one of the most important diseases caused by *Escherichia coli* and It is also considered one of the most common causes of diseases and deaths in children with diarrhea worldwide, especially developing countries [5]. *E. coli* can produce extracellular hemolysin, which is responsible for extra intestinal and Intestinal infections, and is also an important virulence factor [4]. The production of active extracellular α-hemolysin requires the products of the four linked genes *hlyC, hlyA*, *hlyB,* and *hlyD.* A-Hemolysin is synthesized as an inactive polypeptide and converted in its active form by the addition of a fatty acid group catalyzed by the *HlyC* protein. The secretion of α-hemolysin is signal peptide independent and mediated by a specific membrane trans locator system encoded by *hlyB* and *hlyD* [5].PCR assays are proven specific and sensitive in detecting the major virulence genes of *E.coli* therefore the purpose of this study was to detect *hlyA* gene *E.coli* isolated from urine and diarrhea using PCR.

Materials and Methods

Collection of Specimens

In this study, 200 midstream urine specimens were collected randomly from patients presented with urinary tract infections and for both sexes and of different ages and 200 diarrhea specimens were collected from children diarrhea aged less than2 years from AL-Diwaniya Educational, Women, Children and Al Hussein Children's Hospitals in AL-Qadisiyah province, were carried out from 1 April 2016 to 1 November 2017.

An informed consent was obtained from the parent diarrhea and urine collected in a clean sterile container.

Isolation and Identification of *E. coli*

All specimens were streaked onto MacConkey agar (Oxoid, England) and reincubated at 37°C for another 24 h. Pink colonies were selected. And culture and then the bacterial growth were inoculated on Eosin methylene blue agar. Thereafter suspected colonies were streaked onto Blood agar (HiMedia, India) and incubated at 37°C for 24 h hemolytic colonies were selected, and examined for Gram stainability, culture morphological characteristics, and conventional biochemical tests. Identification of E. coli isolates was confirmed by automated identification systems such as api 20E (Biomérieux, France).

Detection hemolysin Production

Plate hemolysis test was done for the detection of haemolysin produced by *E. coli*. The bacteria were inoculated onto 5% human blood agar and sheep blood agar incubated over night at 37 °C.

DNA Extraction

The DNA for 15 bacteria isolates out of 40 *E.coli* isolates was extracted according tothe instruction of the Promega kit. Nano drop spectrophotometer was used for measured the DNA concentration and purity. The extracted DNA was electrophoresed by gel electrophoresis system for proofing that the genomic DNA was intact and not sheared.

Molecular Detection of *hlyA* gene

PCR assay was performed by using specific primer for detection hemolysin toxin gene (*hlyA*). These primers were designed from NCBI-Gen Bank published sequence Escherichia *coli* strain BAU-MH4 *HlyA (hlyA*) gene, partial cds (GenBank: KM596784.1) by using primer3 plus design as in the Table (1).

Table 1: Fragments of hlyA genes primers used in polymerase chain reaction

Primer	Sequence		Amplicon	Source
hlyA- E.coli	F	GGAGTTAGTGCAGCCTCCG	360bp	NCBI Gene Bank
	R	ACCACTCTGACTGCGATCAG		

PCR Master Mixture

Prepare the PCR mixture according to the manufacturer's instructions using the AccuPower ® PCR Pre Mix kit as follows: Table-2.

Table2: Reactants volumes and concentrations used for the PCR amplification of *hlyA* gene

PCR Master mix	Volume (µL)
DNA template	5
Forward primer (10pmol)	1.5
Reveres primer (10pmol)	1.5
PCR water	12
Total volume	20

PCR Thermo cycling Conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in Table-3.

Table 3: PCR program followed to amplify *hlyA gene*

PCR step	Temperature (C°)	Time	repeat
Initial Denaturation	95	5min	1
Denaturation	95	30Sec	30Cycle
Annealing	58	30Sec	
Extension	72	2min	
Final extension	72	10min	1
Hold	4	Forever	_

Results and Discussion

Isolation and Identification of Isolates

Out of 200 urine specimens, 160 (80%) showed culture growth positive distributed to of 102 (73.08%) females and 58(26.9%) males and yielded 76 (47.5%) bacterial

isolates of *E. coli*, and 40 (20%) specimens showed culture growth negative. While diarrhea specimens out 200 specimens 185 (92.5%) showed culture growth positive distributed to of 110 (59.45%) males and 75 (40.54%) females and yielded 80(43.24%) bacterial isolates of *E. coli*. and 15 (7.5%) specimens showed culture growth negative.

Phenotype and Genotype Detection of Hemolysin

The strains of *E.coli* were tested for the ability to produce hemolysins that cause erythrocytes lysis. hemolysin production was detected by determining a zone of lysis around each colony on 5% sheep blood agar plates after overnight incubation. The result showed that out of 76 isolates of *E.coli* of urine only 30 (39.47%) show ability to hemolysis, while The result showed that out of 80 isolates of *E.coli* of diarrhea only 10(12.5%) show ability to hemolysis. The result is agree with [7] which showed that the number and percentage of hemolysin was (41.3%) of *E.coli* from urine. The result is agree with [8] which showed that the number and percentage of hemolysin was (14.9%). of *E. coli* from diarrhea.

Molecular Characterization of hlyA

Fifteen isolated isolates of E. *coli* produced for hemolysin were selected to detect a *hlyA* gene that was divided into 10 isolates of urine and 5 of diarrhea. Polymerase chain reaction technique has been used to amplify gene which encoding the hemolysin enzyme the results clarify that 15 isolates carrying hlyA gene distribution on 10 isolates of urine and 5 isolates of diarrhea. in this study, results revealed that percentage for *hlyA* gene found in *E.coli* isolates of urine 100% and in *E.coli* isolates of diarrhea 100% as in shown in Figure-1 and- 2. Detection of the *hlyA* gene from *E.coli* isolated from the diarrhea for the first time is recorded in Iraq using PCR technique as in Fig-2.

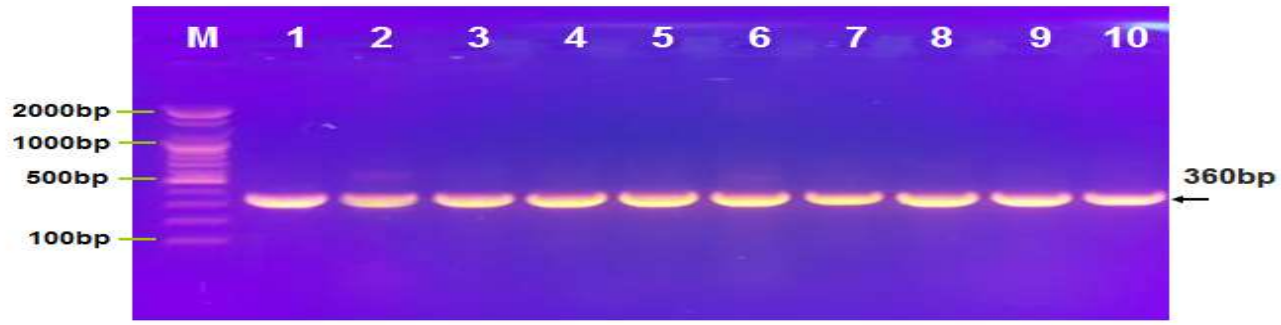

Figure 1: The electrophoresis of the acroose and corneal cells on the results of the PCR examination of the *hlyA* gene in the *Escherichia coli* strain of the urine samples and the 100 voltage for 60 minutes representing M: Marker 2000-100bp and the numbers from (1- 10) represent the positive isolates of the probe with a 360bp length

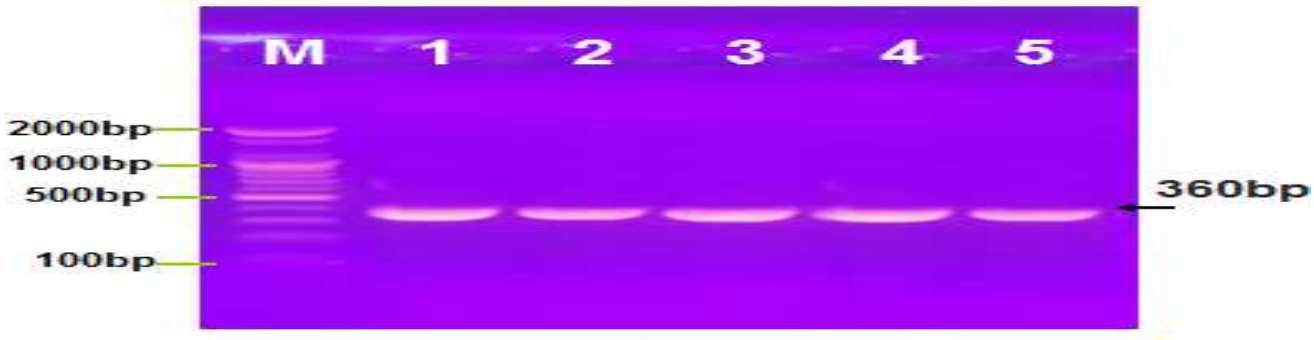

Figure 2: The electrophoresis of the acroose and corneal cells on the results of the PCR examination of the *hlyA* gene in the *Escherichia coli* strain of the diarrhea samples and the 100 mV voltage for 60 minutes representing M: Marker 2000-100bp and the numbers from (1-5) represent the positive isolates of the probe with a 360bp length

Hemolysin a production is the most common toxins produced by *E.coli*, and it was considered virulence factor that isolated from *E.coli*. It has been suggested that the hemolytic strains of *E.coli* was more likely to develop in urinary tract infections, particularly acute pyelonephritis [9]. As that the production of hemolysin contributes to the virulence of the bacteria and favours their proliferation in the intestinal [10].

That hemolysin may play a role in intestinal infections of human with intestinal pathogenic *E. coli*. So, the *α-hly* has been shown to cause cellular damage and trigger inflammation in the mammalian host [11].These results agreed with the results obtained by [12].Who found that the ratio of the presence of a *hlyA* gene in *E.coli* isolates of urine 96%. This study disagrees with [13]. Who found that the ratio of the presence of a *hlyA* gene in *E.coli* isolates of urine a 48% and *E.coli* isolates of diarrhea 15%.

Conclusions

Hlya gene in *E. coli* local isolates are found to exist relatively at a high level among clinical isolates derived from UTI patients and diarrhea, and Found that the bacterium *E. coli* capable of analysis red blood cells for sheep dramatically. In the studies next He suggested the detection of gene expression for *hlyA* gene.

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