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Molecular Detection of *Flu*, Csga Genes of *E. coli* in Apparently Healthy Student in AL-Muthannaa City

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Abstract:

Four hundred midstream urine sample (MSU) were collected from students apparently healthy in ten primary schools in Al-Muthannaa city from the beginning of October 2019 to the end of February 2020. The collected urine were culture on different media (MacConkey agar, Eosin Methylene Blue), and tested biochemically in order to find out the profile of *E.coli*. polymerase chain reaction technique has been used to detect some of virulence genes *Flu, and CsgA* which encoding by *E.coli* isolates. The results demonstrated that out of total 40 isolates (20 urine + 20 stool) there were 25 isolate (62.5%) was positive for *Flu* gene, The prevalence of *Flu* gene in commensal was significantly lower (50%) than that in recurrent higher UPEC (75%). 31 isolate (77.5 %) were positive for *CsgA* gene, The result found a high prevalence (80%) of *CsgA* in UPEC isolates, while *CsgA* gene was detected in (75%) of the intestinal commensal isolates . In this study, describe the profile of *E.coli* bacteria isolated from healthy Iraqi children with no symptoms, for UTI or diarrhea by the identification of virulence genes.

Keywords: Molecular detection, Flu gene, CsgA gene, *E. coli*

Introduction:

Commensal bacteria are an important reservoir of antibiotic resistance genes to pathogenic strains .there was carriage of multiple resistance commensal *E.coli* irrespective of exclusive mode of feeding ,or previous history of use of antibiotic or other parenteral drug .carriage of multiply resistant commensal *E.coli* was present even in infants with no history of use of any drug , there are various environmental and social reasons,(Oluyege *et al* ., 2015).

Young children tend to be the most exposed to antibiotics and several studies have found that the younger children have the highest risk of carrying resistance commensal bacteria (Duerink *et al* ., 2007).The nonpathogenic strains of *E.coli* referred to as commensal strains are harmless and are useful , not only digesting and breaking down food but also in protecting against harmful organisms which may be introduce into the gastrointestinal tract through food and water, (Chinen and Rudensky AY.,2012). However ,such commensal can have access to other part of the body such as urinary tract , blood or wounds and causes opportunistic infection particularly in immune-compromised individual , *E.coli* have been shown to develop resistance in response to antibiotic use and to be particularly capable of exchanging antibiotic resistance genes with Pathogenic bacteria, (Karami *et al* .,2007)

Materials and Methods:

A total of 400 urine samples were collected from school age children (6-12) years apparently healthy during the period from October 2019 to the end of February 2020 .

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The urine of these children were identified by methods of routine tests, colony depending on primary diagnosis , further stool samples were collected from positive *E.coli* urine culture children and cultured for isolation of *E.coli* further detection was done by PCR using *TrpA* gene .

Molecular Detection:

DNA was extracted from activated pure culture of *E.coli* bacteria by using DNA genomic bacteria kit (promega) .detection of DNA band using agarose gel electrophoresis (1%) .Then Conventional PCR were used to detect the important virulance genes (*Flu* , *CsgA*) of *E.coli* bacteria using specific primers shown in table (1) .

Table 1- Sequence of primers of virulence gene.

Gene	Primer sequence 5´---3´	Amplicon size	Refrence
TrpA	5´-GCTACGAATCTCTGTTTGCC_3´	784	(Plantamura *et al*., 2019)
	5´-GCTTTCATCGGTTGTACAAA-3´		
CsgA	For 5´-GATCTGACCCAACGTGGCTTCG-3´	178	(Silva *et al*.,2014)
	Rev 5´-GATGAGCGGTCGCGTTGTTACC-3´		
Flu	For 5´-CGGCGGGCAATGGGTACA-3´	384	(Restieri *et al*.,2007)
	Rev 5-CAGCTCTCACAATCTGGCGAC-3´		

PCR protocol :-

The PCR amplification mixture which used for detection of each gene include FIREPOL® master mix12.5 , 4 µl of DNA template, 1 µl of each forward and reversed primer and 6.5 µl of nuclease free water to complete the amplification mixture to 25 µl table (2).

Table (2):- The PCR mixture components for each gene .

Component	Volume (µl)	Reference
Forward primer	1 µl	(Plantamura *et al*., 2019)
Reverse primer	1 µl	
DNA templet	4µl	
Master mix	12.5µl	
Deionize water	6.5µl	
Final volume	25µl	

After preparing the reaction volume in PCR tube the Mixture was spin down and then PCR tube placed in the PCR thermo cycler and the amplification reaction and condition for each gene was started according to the program described in table (3).

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Table(3):- PCR thermo cycler program for DNA amplification and condition of *E. coli* genes.

Stage	temperature	Time	Number of cycle
Initial denaturation	95c^{o}	5min	1
Denaturation	95c^{o}	30 sec	30
Annealing	x^{o}	30sec	
Extension	72c^{o}	60sec	
Final extension	72c^{o}	5min	1

(X^{o}), annealing temperature for each primer of virulence gene as fellow; *TrpA* , *Flu* , and *CsgA* as 58c ◦55c^{o}, 60c^{o} respectively.

Results and discussion:-

A total of 400 urine sample were collected from school age children (6-12) from October 2019 to the end of February 2020 from both gender. All colonies were diagnosed in the laboratory as *E.coli* bacteria due to characteristic lactose fermenting colonies , daunt shaped and dark pink in color due to reduce pH below 6.8 Figure (1, A),(1,B).



Figure (1):- A-Colonies of *E.coli* on MacConkey shape , B- colonies of *E.coli* on EMB agar .

While the colonies on EMB agar plates appeared metallic shine, which is characteristic for *E.coli* bacteria from other bacteria strains belong to the intestinal family to contain this media eosin and methylene blue dye Figure (1,A,B).

It was found that 64 (80%),(32) sample urine and (32) sample stool of the total samples were positive for culture for *E.coli* were as 16 (20%) negative ,but positive for other type bacteria .the

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extraction of chromosomal DNA was done for(50/64)isolates that diagnosed by vitek 2 system.

The results of gel electrophoresis showed presence of DNA band in the same level in 50 isolate only Figure (2).then the concentration and purity of extraction DNA was measured by Quant flour.



Figure(2): Gel Electrophoresis of genomic DNA extracted from *E.coli* isolates in 1% agarose gel at 75 Volt/Cm for 30 min then visualized under U.V .after staining with Ethid bromide.

Further molecular diagnosis of *E.coli* bacteria by using *TrpA* gene specific primer and the results were detected by electrophoresis 2% agarose and exposed to U.V light in which the results indicates that only 40 isolates give the same results for the biochemical diagnosis Figure (3).

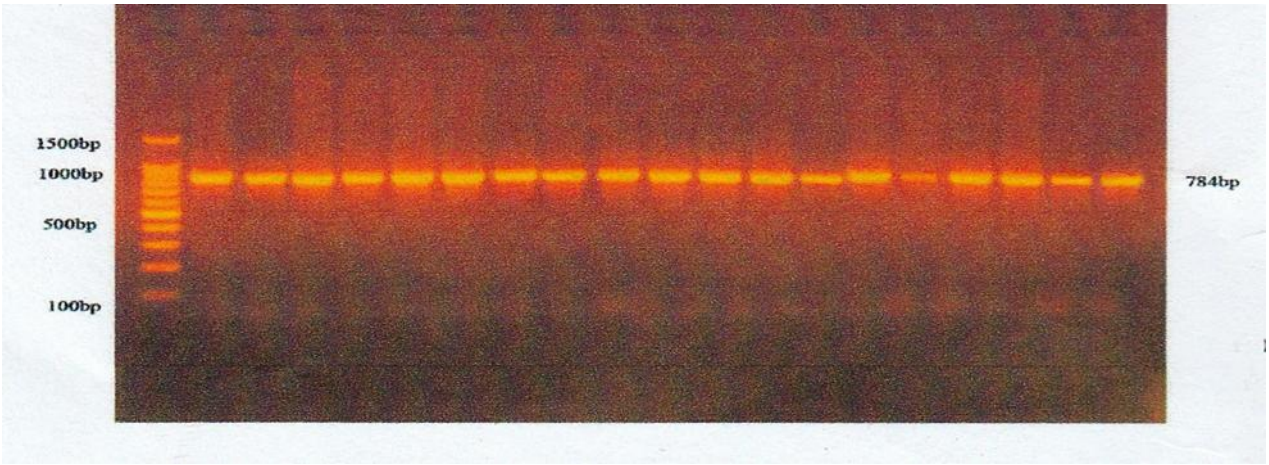


Figure (3): Gel Electrophoresis of PCR amplified products of *E.coli TrpA* gene Lane L: DNA molecular weight Marker (1500pb), Lan (40) positive band at (784 pb) for *TrpA* gene, Lan (10) negative control .

The result revealed that clear bands appeared for *Flu* gene in 25 (62.5%), the prevalence of *Flu* gene in commensal was significantly lower 10(50%) than in UPEC 15 (75%). This gene representive member of the auto transporter family which have functions ranged from cell adhesion to the secreted of toxin Ag43 figure (4).

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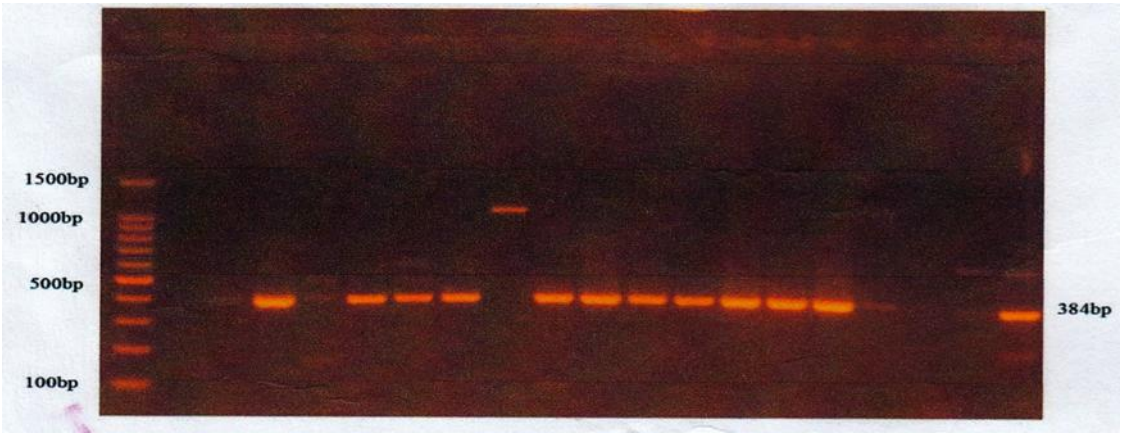


Figure (4):Gel electrophoresis of amplified Flu gene (384bp) from *E.coli* using conventional PCR. Agarose 1%, 70 V/cm for 80 min, stained with ethidium bromide dye and visualized on a UV trans illuminator. Lane (M): 100 pb DNA ladder. Lane (1-15): Amplicon of *Flu* gene.

Furthermore the investigation showed the presence of *CsgA* gene in 31 isolates (77.5%) in a total as shown in Figure (5).

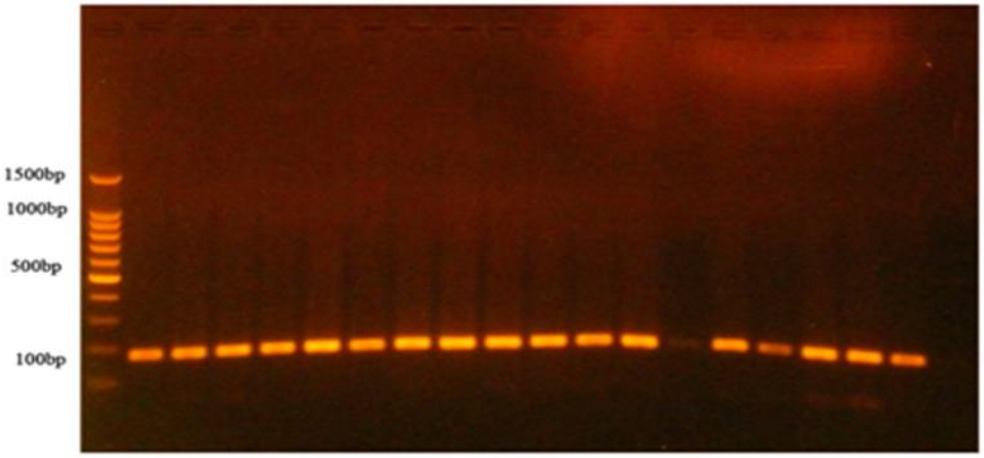


Figure (5) :- Gel Electrophoresis of amplified *CsgA* gene (178 pb) from *E.coli* Agarose 2% , 70 v/cm for 80min , stained with ethidium bromide dye and visualized on UV. trans illuminator . Lan (M) : 100pb DNA ladder.Lan (1-5) : Amplicon of *CsgA* genes .

We found a high prevalence (16/31) of *CsgA* in UPEC isolates , while *CsgA* was detected in (15/31) of intestinal *E.coli* .

The result presented her go a long nicely with the results recorded by Hung *et al* .,(2014) who found that *CsgA* gene presents in 16 (73%) from 22 *E.coli* isolates from children with UTI in India.

Curli fimbria is a fibrous surface protein that is important for biofilm development by *E.coli* and its presence is associated with sever human infection fimbria interacts specifically with host matrix proteins such as fibronectin, laminin and plasminogen to initiate a adherence to and

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colonization of the host Nhu *et al* (2018).

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