Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

Molecular Detection of Flu A and Flu B Genes from Commensal E.Coli Isolation from School Age Children in AL-Muthannaa City

Yahye Mahdi Radi AL- Abady and Dr. Ashwak Bassim Jassim AL-Hashimy

Institute for Genetic Engineering and Biotechnologies, University of Baghdad, Iraq

Abstract:

From the beginning of October 2019 until mid-March of 2020, 400 urine samples were obtained from school children age between of 6-12 years , males and females from 10 schools and various areas in Al-Muthannaa governorate. 10% of the total samples collected by the mean sample method, 40 urine samples for fermentation of lactose sugar appeared on the culture media (MacConkey,EMB), and stool samples were collected from the same people, and they were also positive, only 64 (32 urine + 32 stool) (80 %) samples were confirmed by the vitek 2 system as *E.coli* , while 16 (8 urine + 8 stool) (20%) samples showed another bacteria. 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 , We 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: Flu A gen, Flu B gen, 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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

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 .

The urine of these children were identified by methods of routine tests, colony depending on primary diagnosis , further stool samples were collected 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 in this study 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**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

	Rev 5´-GATGAGCGGTCGCGTTGTTACC-3´		**al.,2014)**
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).

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

Denaturation	95c°	30 sec	30
Annealing	x°	30sec	
Extension	72c°	60sec	
Final extension	72c°	5min	1

X, annealing temperature for each primer of virulence gene as fellow; *TrpA* , *Flu* , and *CsgA* as 58c ◦55c°, 60c° respectively.

Results and discussion:-

A total of 400 urine sample were collected from apparently healthy students from October 2019 to the end of February 2020 from both gender with age ranged from 6-12 years old. 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,B).

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

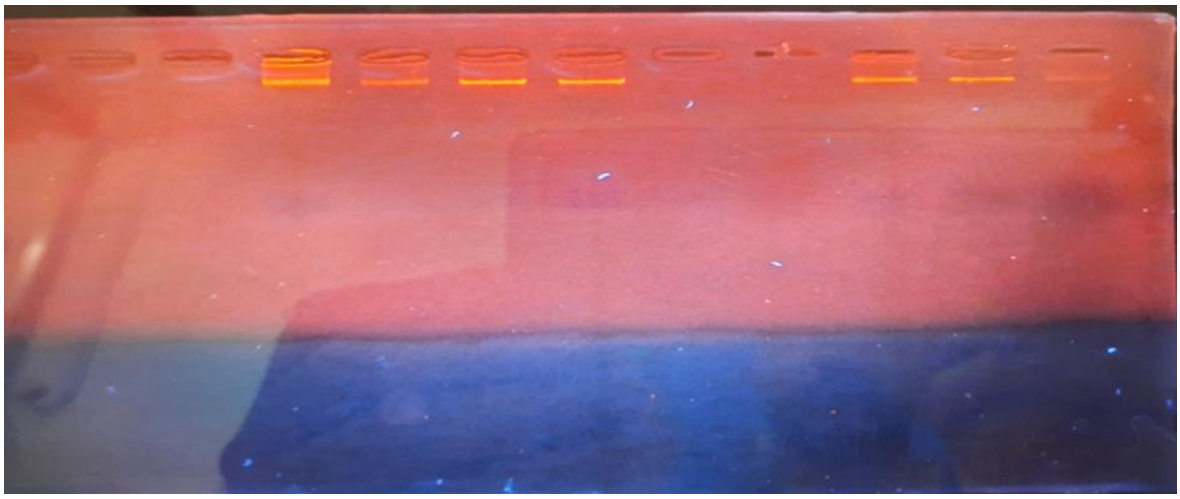
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

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 extraction of chromosomal DNA was done for 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(4-3): 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).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

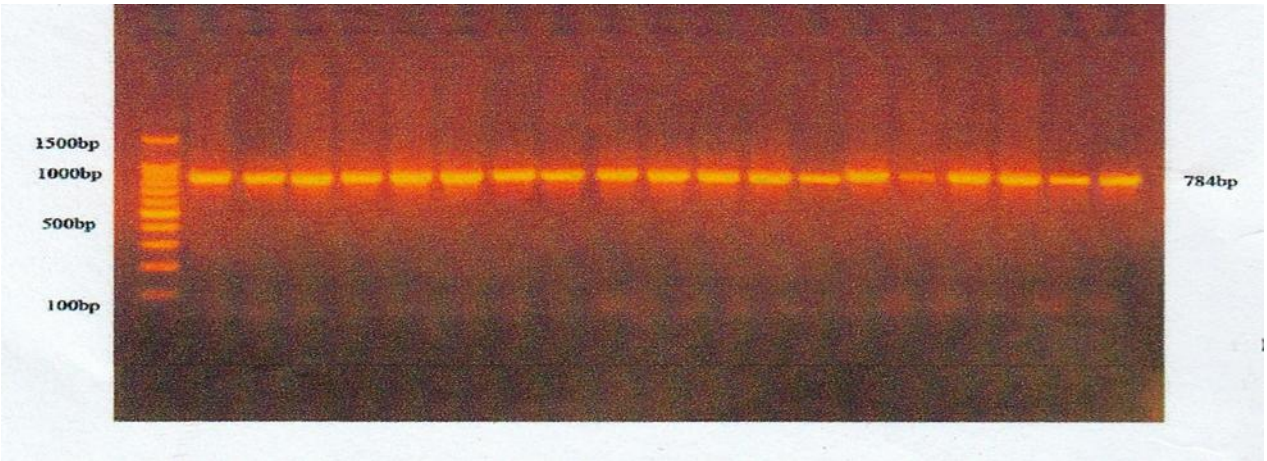


Figure (3): Gel Electrophoresis of PCR amplified products of *E.coli TrpA* gene Lane L: DNA molecular weight Marker (1500pb), Lan (40) positive band at (pb) for *TrpA* gene, Lan (10) negative control .

Approximately all 40 isolate of *E.coli* bacteria gave positive results for flu gene with 384 pb (28) isolates of them include the same student samples (urine , stool) while 12 included different samples which means that the gene appeared figure (4) in isolates from urine or stool only.

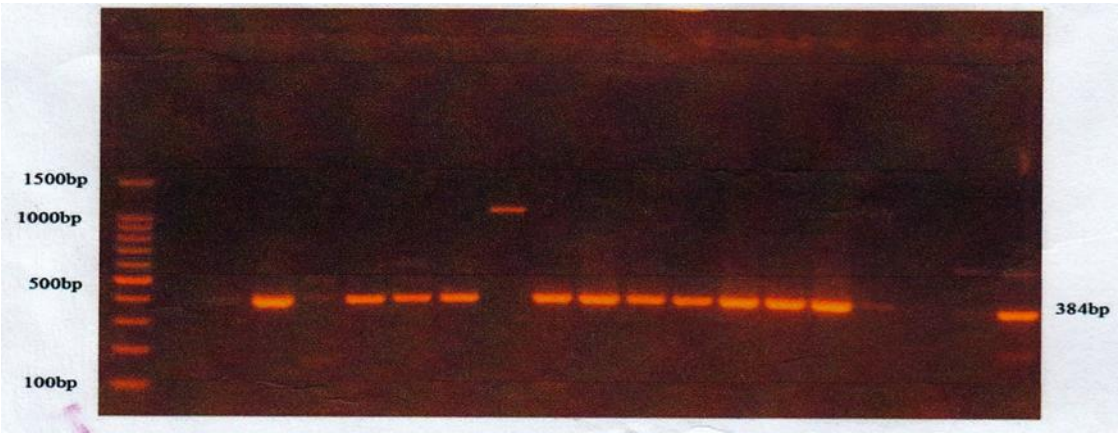


Figure (4-5):Gel electrophoresis of amplified Flu gene (384bp) from E.coli using conventional PCR. Agarose 1%, 70 V/cm for 80 min, stained with ethidium bromide dye and visualized on a UV trans illuminator. Lane (M): 100 pb DNA ladder. Lane (1-15): Amplicon of Flu gene.

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

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.

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

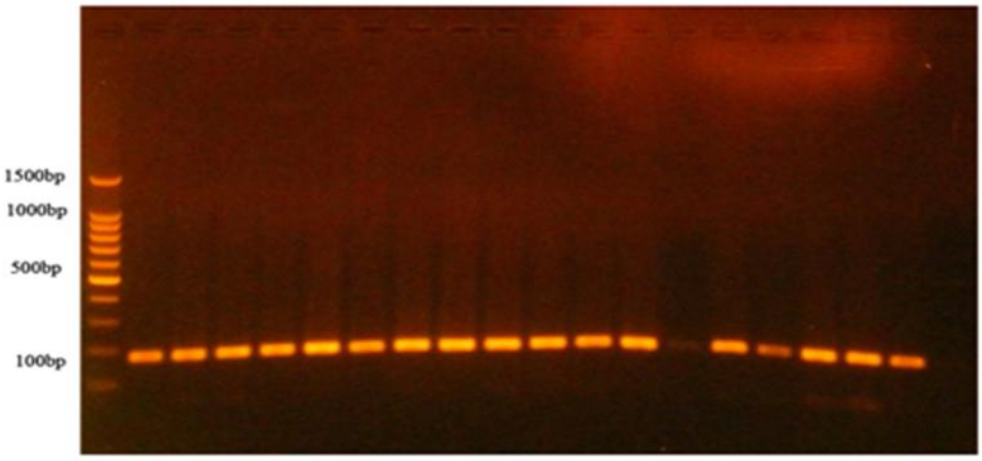


Figure (5) :- Gel Electrophoresis of amplified *CsgA* gene from *E.coli* Agarose 2% , 70 v/cm for 80min , stained with ethidium bromide dye and visualized on UV. trans illuminator . Lan (M) : 100pb DNA ladder.Lan (1-5) : Amplicon of *CsgA* genes(178bp).

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

References

1. **Chinen T ; and Rudensky A.Y.(2012).** The effects of commensal microbiota on immune cell subsets and inflammatory response, Immunol Rev. 2012 Jan;245(1):45-55. doi: 10.1111/j.1600-065X.2011.01083.x.

2. **Duerink D.O ; Lestari E.S ; Hadi U ; Nagelkerke N.J ; Severin J.A ; and Verbrugh H.A, et al(2007).** Study Group Antimicrobial resistance in Indonesia: Prevalence and Prevention (AMRIN): Determinants of carriage of resistant Escherichia coli in the Indonesian population inside and outside hospitals. J. Ant micro Chemotherapy, 2007 (60): 377-384**.**

3. **Hung C ; Marschall J ; Burnham C.A.D ; Byun A.S ; Henderson J.P.(2014).** The Bacterial Amyloid Curli Is Associated with Urinary Source Bloodstream Infection . PLOS one | Volume 9 | Issue 1 | e86009 .

4. **Karami N ; Martner A ; Enne V.I ; Swerkesson S ; Adlerberth I; and Wold A.E.(2007).** Transfer of an Ampicillin resistance gene between two Escherichia coli strains in the bowel microbiota of an infant treated with antibiotics. Journal on Antimicrobial Chemotherapy. 2007. 60 (5): 1142-5.

5. **Nhu N.T.K ; Phan M.D ; Peters K.M ; Lo A.W ; Forde B.M;and Chong T.M. ; et al .(2018).** Discovery of New Genes Involved in Curli Production by a Uropathogenic Escherichia coli Strain from the Highly Virulent O45:K1:H7 Lineage. mBio Journal, Volume 9 Issue 4 e01462-18.

6. **Oluyege A.O ; Ojo-Bola O ; and Oludada O.E.(2015).** Carriage of Antibiotic Resistant Commensal E. coli in Infants below 5 Months in A do-Ekiti, Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 1096-1102**.**

7. **Plantamura J ; Bousquet A ; Védy S. S ; Larréché S ; Bigaillon C ; and Delacour H ; et al . (2019)** . Molecular epidemiological of extended-spectrum β-lactamase producing Escherichia coli isolated in Djibouti. J Infect Dev Ctries . 2019 Aug 31;13(8):753-758. Doi: 10.3855/jidc.11283. PMCID: PMC3890832 PMID: 24335802Microbiolog.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 2497 – 2505
Received 05 March 2021; Accepted 01 April 2021.

8. **Restieri C ; Garriss G ; Institutet K ; Locas M.C ; and Dozois M.C (2007).** Autotransporter-Encoding Sequences Are Phylogenetically Distributed among Escherichia coli Clinical Isolates and Reference Strains, April 2007Applied and Environmental Microbiology 73(5):1553-62 DOI:10.1128/AEM.01542-06.

9. **Silva V.O ; Soares L.O ; Júnior A.S ; Mantovani H.C ; Chang Y. ; and Moreira M.S. (2014) .** Biofilm Formation on Biotic and Abiotic Surfaces in the Presence ofAntimicrobials by Escherichia coli Isolates from Cases of BovineMastitis. Applied and Environmental Microbiology 80(19).