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Molecular Detection of the *fim*H Gene and Studying the Effect of apigenin on Colonization Factors for Clinical Isolates of *Escherichia coli*

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التشخيص الجزيئي لجين *fim*H ودراسة تأثير الابجينين على عوامل الاستيطان لعزلات سريرية من الاشريشية القولونية

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ABSTRACT

Background:

Escherichia species, which include *E. fergusonii*, *E. vulneris*, *E. hermanii*, *E. blattae*, *E. albertii*, and *E. coli*, are members of the bacterial family Enterobacteriaceae.One of the most crucial components of *E. coli's* pathogenicity is adhesion, and the invasion process is thought to begin when the bacteria bind to uroepithelial cells. Numerous *E. coli* strains express type 1 fimbria (pilus), which is present in more than 95% of *E. coli* isolates from infections inside and outside the body. For bacteria that live in a variety of settings, including biotic and abiotic surfaces, pili function as highly effective adhesion tools. The distribution of type 1 pili on the surface of UPEC is uniform.

Materials and Methods:

For this study, (80) samples were gathered from patients with various ailments who visited hospitals in the AL margan Hilla city between July and October 2022. The samples came from individuals of both sexes and of various ages.The patients' ages ranged from 20 to 60.

Results:

18 *E. coli* isolates were found in this study among patients with UTI, vaginitis, and wound infections.

By employing PCR methods, the results revealed that only 14 isolates were positive for the *fim* H gene.

Conclusion

Most commonly, urine, vagina, and wounds can be used to isolate *Escherichia coli*. The *fim* H gene, which is important for bacterial adhesion, was present in the majority of *E. coli* isolates. The PCR method is used to find some sticky genes. All *E. coli* clinical isolates tested positive for colonization factor antigen (CFA). On CFA, epigenine had little impact.

Key words:

E.coli, molecular detection *fim* H gene ,colonization factor antigen,Apigenine.

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INTRODUCTION

The bacteria in the Enterobacteriacae family is *Escherichia coli*. This bacteria can digest lactose in Macconky agar and is either aerobic or facultatively anaerobic.[1] However, type 1 filaments are notably real virulence components of UPEC that can enhance *E. coli's* attachment to several cell types[2].

The majority of *E. coli* strains, including Enterotoxigenic *E. coli* (ETEC), which can create a variety of adhesive factors, including colonization factor antigen (CFA), which has been previously documented in the majority of commensals, and Pathogenic *E. coli*, may also produce pilli or fimbriae[3].

Apegenine, a molecule produced from numerous plants and found to have an effect on *Fim* H by molecular docking[4], is one of the various chemical and herbal substances that may play a role in influencing pill function.

Additionally, a variety of mobile elements, such as plasmids, phages, integrons, and pathogenicity islands, may contain one or more adhesive factors as well as genes encoding one or more virulence factors, such as colonization factors and secretion types[5].

2. MATERIALS AND METHODS:

For this study, (80) samples were gathered from patients with various diseases who visited hospitals in the AL margan Hilla city between July and October 2022. The samples came from individuals of both sexes and of various ages.The patients' ages ranged from 20 to 60.

Collection of samples: For this experiment, DNA was extracted from 15 samples of urine, vaginal swabs, and wounds obtained from E. coli patients, and the samples were then cultured on Macconky media.

Specimen Collection:

Below is a description of the proper samples to be obtained for bacteriological analysis. The right procedures were followed when collecting such samples in order to prevent contamination.[6].

1.Urine samples:

The patients who provided the samples typically had UTIs. In sterile screw-cap containers, midstream urine samples were taken.

2- vaginal swabs:

Typically, vaginitis patients provided the specimens for collection.

3- wound swabs:

Typically, patients with wound infections provided the specimens for collection.

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Laboratory Diagnosis

Bacterial Identification Assay:

The isolation and identification of E. coli linked to study patients were carried out in accordance with the diagnostic techniques advised by[7] as follows:

Colonial Morphology and Microscopic Examination

Each main positive culture produced a single colony. The morphology characteristics (colony size, form, color, translucency, edge, and elevation of texture) determine its identity.

Gram stain was then used to look into the colonies and look at the bacterial cells. To get at the ultimate identification, specific biochemical tests were conducted.

On culture media (EMB), the E coli had the following characteristics: circular shape, whole edge, elevated, tiny size, smooth texture, and green shining appearance.

Biochemical Tests[8].

1-Catalase Test:

An enzyme called catalase helps hydrogen peroxide release its oxygen. The chosen bacterial colonies were streaked over nutrient agar medium, which was then incubated at 37°C for 24 hours. The growth was then transferred onto a clean slide by using a wooden stick, and a drop of (3% H2O2) was then applied. A successful outcome was suggested by the formation of gas bubbles [9].

2-Oxidase Test

The test relies on the presence of specific bacterial oxidases that would facilitate the transfer of electrons between the bacteria's electron donors and a redox dye (tetramethyl-phenylene-diamine-dihydrochloride), which was reduced to a deep purple hue.

The colony to be tested was picked up with a sterile wooden stick and smeared on a strip of filter paper that had been soaked in some freshly prepared reagent.

An intense deep purple tint that developed on the filter paper within 5–10 seconds was a sign of a successful filtering operation[10].

3-Indole test:

For this test, a peptone water medium with bacterial growth via the loop was infected, and it was incubated for 24 hours at 37 °C.

By putting 6–8 drops of Kovac's reagent (p-dimethyl amino Benzaldehyde in amyl alcohol) into the indole, the test was conducted. The development of a red color ring at the top of the soup was indicative of a favorable reaction[8].

4-Methyl-red test:

Selective bacterial colonies were used to inoculate the MR-VP broth tubes, which were then incubated for 24 hours at 37°C. Then, five drops of the methyl red reagent were added. Red color's emergence and observation indicates a successful outcome and full glucose hydrolysis [8].

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5-Vogues – Proskauer test (VP)

Selected bacterial colonies were used to inoculate the MR-VP broth tubes, which were then incubated for 24 hours at 37°C. After that, the outcome was read by adding 4 drops of 40% KOH solution and 5–12 drops of alpha nephthol (reagents A and B). Due to the partial hydrolysis of glucose, which resulted in acetoin or acetyl - methyl - carbinol, the development of red hue after 15 minutes to 1 hour indicates a successful outcome [8].

6-Citrate utilization test

The bacterial colonies were inoculated and then cultured for 24 hours at 37°C following the autoclave sterilization of Simmons Citrate slants. The media's transition from green to blue showed that the organisms could use citrate as their only carbon source [11].

7-Urease test

By inoculating urea medium with bacterial growth, this test was conducted. 24-48 hours were spent incubating the tubes at 37 °C. A successful outcome was established when medium turned pink[11].

Extraction of genomic DNA from *E.coli* isolates:

Utilizing a genomic DNA kit provided by the manufacturer (Bioneer/Korea), DNA was extracted from bacterial cells.

Each isolate was cultured and inoculated into 5 ml of Brain Heart Infusion (BHI), where one colony of each was developed overnight at 37 °C. by means of these isolated cultures. All PCR tests used the acquired DNA as templates. Using certain primer pairs as specified, the target DNA was amplified by PCR (polymerase chain reaction) (Table 1). A PCR product (amplicon) is created by repeating three phases back-to-back for a predetermined number of cycles. This amplicon may be seen following agarose gel electrophoresis, which reveals information about the thermal cycling conditions.

Table (1): The PCR primers with their sequence and amplicon size:

Primers	Sequence		Product Size
fimH genes	F	5'- ATGAAACGAGTTATTACCCT-3'	903bp
	R	5'- TTATTGAT AAACAAA AGTCAC-3′	

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Initial preparation process

According to the manufacturing company's instructions (Bioneer, Korea), the upstream and downstream primers were manufactured and stored at -20oC.

Using DNA rehydration solution 1X (pH 8.0) Tris-EDTA buffer (TE-buffer), lyophilized primer pairs were rehydrated. The working solution would be created from the primer stock tube after the primer storage-stock tube was prepared. To create a primer stock solution with a 100 picomole/microliter concentration, TE buffer was added in accordance with the producer's (Bioneer/Korea) specifications. To obtain 10 picomoles per microliter, the working solution was diluted with TE buffer to a ratio of 1:10 (v/v) from stock.

Electrophoresis of agar:

In order to make the gel, agarose powder was mixed with 1X TBE buffer, dissolved by boiling, and then allowed to cool to 50oC. Depending on the purpose for which agarose is employed, a different amount of agarose powder was dissolved.

1% agarose is used to see the DNA following extraction for DNA profiling. While 1.5% agarose was used to visualize the PCR product (amplicon), 3% agarose is utilized to detect single nucleotide polymorph

Ethidium bromide stock solution with a concentration 10mg/ml was used. Only 5µl of this stock solution were supplemented to 100ml of melted agarose gel to get final concentration.

Table (2): Cycling parameters of *Fimh* gene amplification

CycleNo.	Stage	Temp.°C	Time	Reference
1	Initial denaturation	**95**	3 min.	[13]
35	Denaturation	**94**	40 sec.	
	Annealing	**42**	45 sec.	
	Elongation	**72**	1 min.	
1	Final Extension	**72**	5 min.	
1	Final hold	**4**	------	

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Ethical certification:

1-The necessary ethical approval was obtained by verbal consent from patients.

2-This study was approved by the committee of publication ethics at College of Medicine, Babylon Province, Iraq.

RESULTS AND DISCUSSION:

Isolation of *Escherichia coli* :

Among 80 clinical samples,50 samples were positive bacterial growth, 30 samples were positive for E coli and 20 positive for other organisms ,15(42.8%) isolates *E.coli* were obtained from 35 urine samples ; 10 (40%) isolates from 25 samples of vagina ; 5(25%) isolates from 20 samples of wound.

Table (3): Distribution of *E. coli* isolates from clinical samples

Sources of isolates	No. of samples	No. of *E. coli* isolates
Urine	35	15(42.8%)
Vagina	25	10(40%)
Wound	20	5(25%)
Total number	80	30(37.5%)

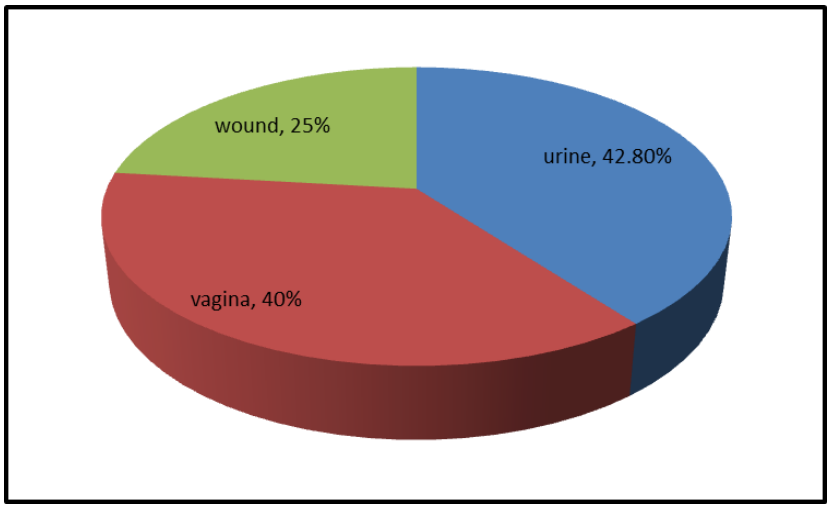

Figure (1)

Table (4): biochemical test of *E.coli*

Characteristics	Test result of *E.coli*
CAMP	–
Capsule (Capsulated/Non-Capsulated)	Variable
Catalase	Positive (+ve)
Citrate	Negative (-ve)
DNase	Negative (-ve)
Gram Staining	Negative
H2S	Negative (-ve)
Hemolysis (Alfa/Beta/Gamma)	Some Strains shows Hemolysis
Indole	Positive (+ve)
Lactose fermentive	Positive (+ve)
Lysine decarboxylase	Positive (+ve)
MR(Methyl red)	Positive (+ve)
Nitrate Reduction	Positive (+ve)
Motility (Motile/Non-Motile)	Motile
Oxidase	Negative (-ve)

Colonization Factor Antigen(CFA):-

A- Detection of (CFA/I):-

After culturing the organism on brain heart infusion broty and incubating it for 24hr. at 37°C, the agglutination of RBC with bacteria occurs in presence of D-mannose as follows:-

1-RBC suspension is prepared from the human blood (group A) and washed with phosphate buffer saline (repeated 3 times). 3% suspension from RBC(v/v) is then prepared.

2- A bacterial suspension is prepared by taking half of the bacterial growth from brain heart infusion broth and mixing it with 1ml of buffer saline to determine RBC agglutination test and vasticated colonization factor antigen type1.

3- On a clean slide, one drop of bacterial suspension is mixed with one drop of 0.1M D-mannose on one side, and with one drop of 3% suspension,and on the other slide one drop of bacterial suspension is mixed with one drop of 0.1M D-mannose on one side with one drop of 3% suspension ,and one drop of apigenine.

The agglutination of RBC with bacteria is detected after 1-2 min in room temperature which is considerd positive.[14].

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B-Effect of epigenine on CFA:

According to the steps done at (A).Epigenine was prepared at different concentration (0.1%,0.2%,0.5% and 1%) and observed its effect on CFA.

Only one drop of epigenine solution was added to the mixture of CFA solutions to show if there was agglutination or not.

[15]reported that *E coli* isolates were identified using biochemical tests and were screened by PCR. The *fim* H gene was amplified using specific primers and showed a band about 903bp. The fimH gene was found in 14 isolates of the *E.coli* strains. Of 30 isolates positive for the *E. coli.*

[16] reported that Type 1, or mannose-sensitive, fimbriae are produced by >80% of all *E. coli*. It is now well established that the expression of type 1 fimbriae by *E.coli* is a virulence factor for pathogenesis of the urinary tract.[17] have been reported that more than 95% of all *E. coli* isolates express type 1 fimbriae . The type 1 pilus is 2 µm in length and 10 nm in width, and is highly represented in the bacterial surface (100–500 pili per cell) . This pilus is defined as mannose-sensitive, because it is able to interact with the mannosylated receptors expressed by epithelial cells, particularly urothelial cells .This specific function relies on the expression of the adhesion *fim*H located at the tip of the type 1 pilus [18] when observing the presence of the adhesin *fim*H in the total isolates of *Escherichia coli* in urine cultures of isolates.

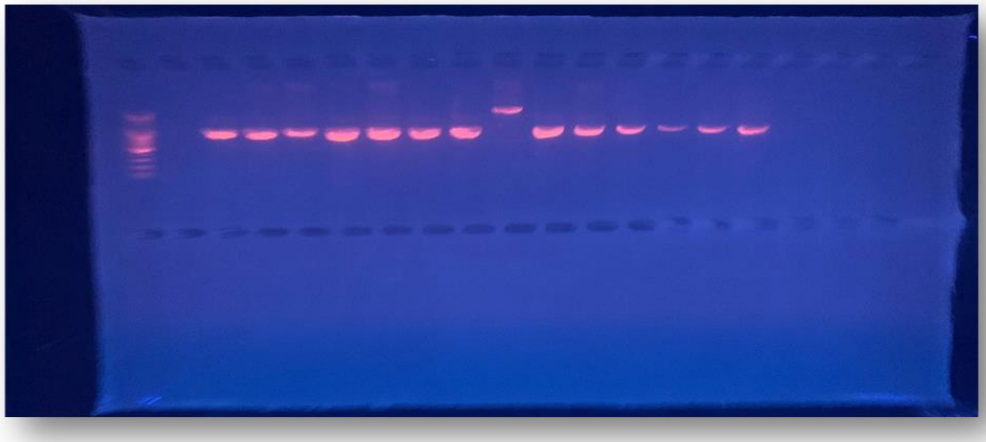

Figure (2) Detection of colonization factor antigen(CFA) by *E.coli* isolates:

Colonization factor antigen (CFA) was investigated in all *E.coli* isolates.The method of detection showed that all clinical isolates of this bacteria gave positive results of agglutination in the presence of mannose sugar.This positive results mean that this bacteria may have different colonization factor that give rise to the ability of bacteria to adhere different types of tissues at various rates where the bacteria seems to show resistance to mannose sugar which is one of the most important sugar in the structure of numerous tissues in the human.

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Epigenine is also used in this study to show its affect on CFA at various concentrations .The results showed that Epigenine has no effect of CFA where there is no effect on agglutination results of CFA detection.

DISCUSSION

In this study, This table indicates that *E. coli* are highly isolated from urine samples, followed by vaginal swabs samples, and then wound samples. These results are identical with [19]. Who confirmed that *E.coli* was predominant in urinary tract infection[20].

Although this bacteria is the normal flora of the gut in human but only 5 isolates were found in wound samples[21]and [22].

In this study, *fim*H gene is detected in all *E.coli* isolates (30 isolates) by using PCR technique, Where specific primer is used for this study.

The results found as shown figure (3-2) that only 14 isolates gave positive results for the presence of *fim* H gene where as this gene is not observed in other isolates.

Epigenine may have no effect of CFA because colonization factor antigen may not include in its structure the *fim* H protein, where the later has strongly influenced by Epigenine or showed by molecular docking done by previous study conducted in Babylon province by [4].

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Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

تنتمي أنواع الاشريكية القولونية الى العائلةالبكتريا المعوية وهي *E. fergusonii, E. vulneris, E. hermanii, E. blattae, E. albertii* و *E. coli* . يعد الالتصاق أحد أهم عوامل الضراوة في بكتيريا الإشريكية القولونية، ويعتبر التصاق البكتيريا بالخلايا البولية الظهارية الخطوة الأولى للاختراق. يتم التعبير عن نوع 1 الخمل (شعيرات) من خلال عدد كبير من سلالات الإشريكية القولونية، وتوجد في أكثر من 95% من عزلات الإشريكية القولونية من التهابات الأمعاء وخارج الأمعاء. تعمل الخمل كأدوات التصاق عالية الكفاءة للاستيطان البكتيري في بيئات متنوعة، بما في ذلك الأسطح الحيوية وغير الحيوية. يعد النوع 1 من الخمل بشكل واسع مسبب للامراض البولية.

طرق العمل:

شملت هذه الدراسة (80) عينة تم جمعها من مرضى يعانون من أمراض مختلفة ، العينات التي تم جمعها من كلا الجنسين والأعمار المختلفة ، الذين حضروا إلى مستشفيات مدينة المرجان الحلة ، خلال الفترة من يوليو إلى أكتوبر 2022 ، وتراوحت أعمار المرضى من 20 إلى 60 عاما.

الاستنتاجات:

1- الإشريكية القولونية هي في الغالب يمكن عزلها عن البول والمهبل والجروح.

2-يتم الكشف عن بعض الجينات اللاصقة باستخدام تقنية تفاعل البوليميرات المتسلسل.

3-يوجد جين *fim* H في معظم عزلات الإشريكية القولونية.

4-كان مستضد عامل الاستيطان (CFA) إيجابيا في جميع العزلات السريرية للإشريكية القولونية.

5- الابجنين ليس له أي تأثير على CFA

الكلمات المفتاحية: الإشريكية القولونية, تفاعل البوليميرات المتسلسل,*fim*H,الابجنين,مستضد عامل الاستيطان.