Pathogenicity islands of *Escherichia coli* isolated from clinical samples

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الخلاصة

الجزر المرضية توجد على الكروموسوم البكتيري, ومع ذلك توجد ايضا على البلازميد البكتيري. وقد تم اكتشاف الجزر المرضية لبكتريا القولون المعوية التي تصيب الجهاز البولي.
الكشف عن بعض الجزر المرضية لبكتريا القولون المعوية المعزولة من عينات سريرية مختلفة.
تم دراسة اثنا عشر عزلة من البكتريا المعوية معزولة من عينات سريرية مختلفة شخصت بالطرق البكتريولوجية. وقد استخدم جهاز PCRللكشف عن بعض انواع الجزر المرضية. وقد استخدم جهاز الترحيل الكهربائي بفولطية60 ولمدة 45 دقيقة.
في هذه الدراسة اثنا عشر عزلة من البكتريا المعوية خضعت للدراسة الوراثية للكشف عن وجود بعض الجزر المرضية باستخدام جهاز PCRوقد اظهرت الدراسة بان الجين *PAi* موجود في عزلة واحدة فقط وهي الادرار. وقد لوحظ ايضا ان *papGII* موجودة في عشر عزلات اغلبها تعود الى عزلات الادرار والمسحات المهبلية بينما *papGI* غير موجود في جميع العزلات. كما لوحظ ان *papC* موجود في عزلة واحدة فقط هي الادرار. من عوامل الالتصاق الاخرى هي *sfa* وقد لوحظ وجودها في ثلاثة عزلات فقط. وقد اظهرت الدراسة ايضا الهيمولايسين موجود في خمس عزلات اغلبها تعود الى عزلات الادرار وقد وجد ايضا ان لهذه العزلات لها القدرة على انتاج الهيمولايسين في الوسط الزرعي. وقد لوحظ ايضا ان جين *iucC* موجود في ثمان عزلات تعود اغلبها الى مسحات المستقيم وعزلات الخروج.
كل خطوة من عملية الاصابة تكون بواسطة مجموعة من عوامل الضراوة, وكل عزلة تمتلك مجموعة من هذه العوامل.

Abstract

Background:- Pathogenicity islands (PAI) are located on the chromosome; however, they can also be part of bacterial plasmid and phages . PAIs were first described in uropathogenic *E. coli* (UPEC).
Gene encoding for hemolysin, P pili, S fimbriae have been found on various PAIs in different *E. coli* strains of UTI .

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Objective:- The aim of this study to investigate the some of pathogenicity islands markers of *E.coli* isolated from clinical samples by using molecular primer.
Patients and methods:- In a total, 12 *E.coli* strains were collected in this study. Strains were isolated from various clinical samples by standard bacteriological methods. PCR was conducted to determine the PAIs of the isolates by using primers *papC, papG(I, II)*, *sfa, hlyC/A* , *iucC* and *PAi*. The PCR amplification product were visualized by electrophoresis on 1% agarose gels for 45min at 60v. the size of the amplicons were determined by comparison to the 100 bp allelic ladder.
Results: In this study, 12 isolates of *Escherichia coli* were genotypically characterized by PCR assay. Among isolates studies it was found that *PAi* is found in only one isolates (urine). *PapGI* is absent from all isolates while *papGII* is present in ten isolates mostly in urine (n=3) and vagina(n=3), *papC* is present in only one isolate include urine, *sfa* is present only in three isolates. Also, the gene *hlyC/A* is present in five isolates, mostly in urine(n=3), also, these positive isolates had the ability to produce extracellular hemolysin, and aerobactein gene *iucC* is present in eight isolates mostly in stool(n=3) and rectal(n=3).
Conclusion: Each steps in the infection process can be mediated by a number of alternative virulence factors and each strain may have a unique combination of these factors**.**
Recommendation:- Direct molecular identification of *E. coli* in clinical samples.

Introduction

Escherichia coli is the most abundant facultative anaerobe of the human intestinal microflora. Despite the fact that it is a commensal bacterium, some *E. coli* strains have acquired specific virulence attributes that confer an increased ability to adapt to new niches and allow them to cause a broad spectrum of disease either intestinal or extraintestinal sites (1). Often the virulence attributed are genetically linked located I a subgroup of genomic islands, in the so called pathogenicity islands (PAI) (2).

PAIs are located on the chromosome; however, they can also be part of bacterial plasmid and phages (3). PAIs were first described in

uropathogenic *E. coli* (UPEC). Gene encoding for hymolysin, P pili, S fimbriae have been found on various PAIs in different *E. coli* strains of UTI (4).

P-pili, the adhesins most clearly implicated in the pathogenesis of extraintestinal infection due to *E. coli*. It encoded by the pyelonephritis-associated pili (*pap*) genes, are significantly prevalent among strains of UPEC that cause pyelonephritis (5) and are characterized by their mannose-resistant adherence to Galα1 4Galβ disaccharide, confer binding not only to urinary tract epithelial cells but also to colonic epithelium. The P fimbrial adhesin molecule PapG recognizes globoseries of glycolipids as receptor (4). PapG occurs in three recognized variants, termed class I, II, and III. The class I adhesion binds globotriaosylceramide (GbO3), class I papG, not much is known about its clinical association (6), while class II recognizes globoside (GbO4), it is more often found in pyelonephritis and bacteremia in human (7), and class III the Forssman glycolipid (GbO5). The *papC* gene encodes the outer membrane usher protein that is required for ordered P fimbriae assembly (8). Many studies showed that P fimbriae occur more frequently among UPEC than fecal isolates.

S fimbriae bind to sialy galactosides. An important observation is that S fimbriae occur in some pyelonephritogenic *E. coli* strains but are mainly associated with strains causing neonatal sepsis and meningitis (9).Also, other virulence gene is α-hemolsin (HlyA), which is associated with both intestinal and extra-intestinal infection. Four genes (*hlyA, hlyB, hlyC, and hlyD*) are required for the production and export of the hemolysin (10).HlyA is produced by most hemolytic *E. coli* strains isolated from clinical material (11). So, the HlyA is a member of RTX toxin family.Aerobactin, a bacterial siderophore, has recently been shown to be associated with *E. coli* strains which cause pyelonephritis and cystitis. Aerobactin has long been known as an important hydroxamate iron uptake system of pathogenic and non-pathogenic *E. coli*. The biosynthesis of aerobactin is encoded by four genes (iuc*ABCD*) (12). It is an iron sequestration and transport system which enables *E. coli* to grow in iron poor environments such as dilute urine and complement- depleted serum (13).

Materials and Methods

Patients:

Twelve *E. coli* isolates were collected in this study, which were isolated from various clinical samples by standard bacteriological methods. From 12 *E. coli* strains, 3 were isolated from urine sample from patients with urinary tract infection, vaginal strains comprised 3 genital tract (cervix or vagina) isolated from women suffering from vaginitis, rectal strains (n=3) were isolated from were isolated from the rectum of women. Also three samples of stool swab isolated from patients complaining from diarrhea . All samples were obtained from patients or individuals who admitted to Babylon Hospital for Maternal and Pediatrics, and to Al-Hilla Surgical Teaching Hospital in Babylon city in addition to swabs taken from private clinics during the period from February 2010 to May 2010.

Bacterial culture:

The samples were processed on MacConkey and Eosin methylene blue agar and were incubated at 37c overnight. The identification of gram negative bacteria was performed by standard biochemical methods (catalase test, oxidase test, and IMViC test) according to Bergy's Manual for Determinative Bacteriology (14).

DNA extraction for gram negative bacteria:

This method is made according to the genomic DNA purification kit supplemented by manufactured company (promega, USA) (cat#A 1120).

Detection of some pathogenicity islands markers by PCR:

The primers and PCR conditions used to amplify genes encoding virulence genes with PCR are listed in table (1). The primers for the P fimbrial *papC* gene and for S fimbriae *sfa* gene, as well as the primer specific for the two *papG* adhesion varieties class I, and class II. For aerobactin and hemolysin, we used primers amplifying DNA of the *iucC* and *hlyC/A* genes respectively. Finally, pathogenicity associated islands marker encode by *PAi* gene. Each 25µl of PCR reaction mixture for PCR contained 2.5µl of upstream primer, 2.5µl of downstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of master mix. The PCR amplification product were visualized by electrophoresis on 1% agarose gels for 45min at 60v. the size of the amplicons were determined by comparison to the 100 bp allelic ladder (promega, USA) (cat# G 2101).

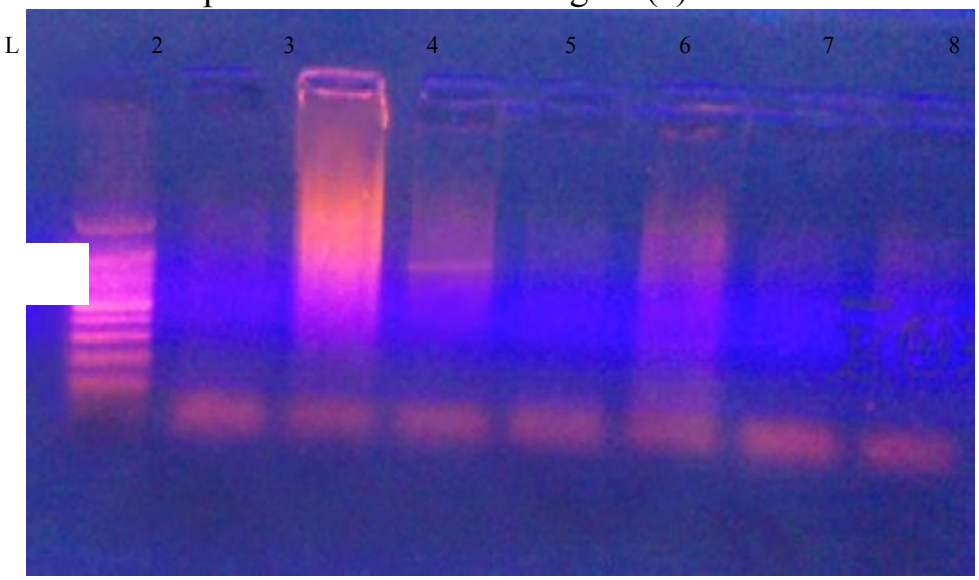
Table (1):- Primers sequences and PCR condition to detect PAIs

Genes	Primer sequence (5□-3□)	Size of product bp	PCR condition	Refrence
papC F *papC* R	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	382	94ºC 3min 1x 94ºC 2min 65ºC 1min 28x 72ºC 1min 72ºC 10min 1x	15
papGI F *papGI* R	TCGTGCTCAGGTCCGGAATTT TGGCATCCCACATTATCG	461	94ºC 5min 1x 94ºC 1min 55ºC 1min 30x 72ºC 1min 72ºC 10min 1x	16
papGII F *papGII* R	GGGATGAGCGGGCCTTTGAT CGGGCCCCCAAGTAACTCG	196	95ºC 2.5min 1x 94ºC 30sec 55ºC 1min 30x 72ºC 30sec 72ºC 7min 1x	17
sfa F *sfa* R	CTCCGGAGAACTGGGTGCATCTTAC CATCAAGCTGTTTGTTCGTCCGCCG	410	94ºC 3min 1x 94ºC 2min 65ºC 1min 28x 72ºC 1min 72ºC 10min 1x	15
iucC F *iucC* R	AAACCTGGCTTACGCAACTGT ACCCGTCTGCAAATCATGGAT	269	94ºC 3min 1x 94ºC 1min 55ºC 1min 30x 72ºC 1min 72ºC 7min 1x	18
hlyC/A F *hlyC/A* R	AGGTTCTTGGGCATGTATCCT TTGCTTTGCAGACTGCAGTGT	556	94ºC 5min 1x 94ºC 30sec 55ºC 30sec 30x 72ºC 30sec 72ºC 7min 1x	18
PAi F *PAi* R	GGACATCCTGTTACAGCGCGCA TCGCCACCAATCACAGCGAAC	930	94ºC 4min 1x 94ºC 1min 55ºC 1min 30x 72ºC 1min 72ºC 7min 1x	16

Company of primers is alpha (USA).

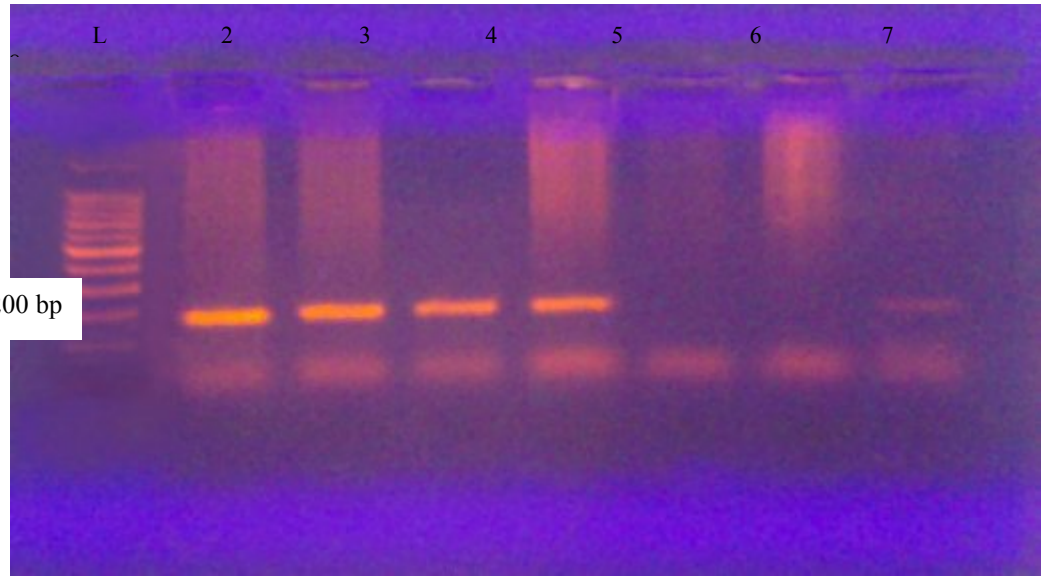
Results

PCR was used to investigate the presence of *papG(I,II), papC, sfa, hlyC/A, iucC, and PAi* genes of 12 *E.coli* isolated from clinical samples. It was found that *PAi* is present only in one isolate, include urine are positive after amplification is shown in figure (1).



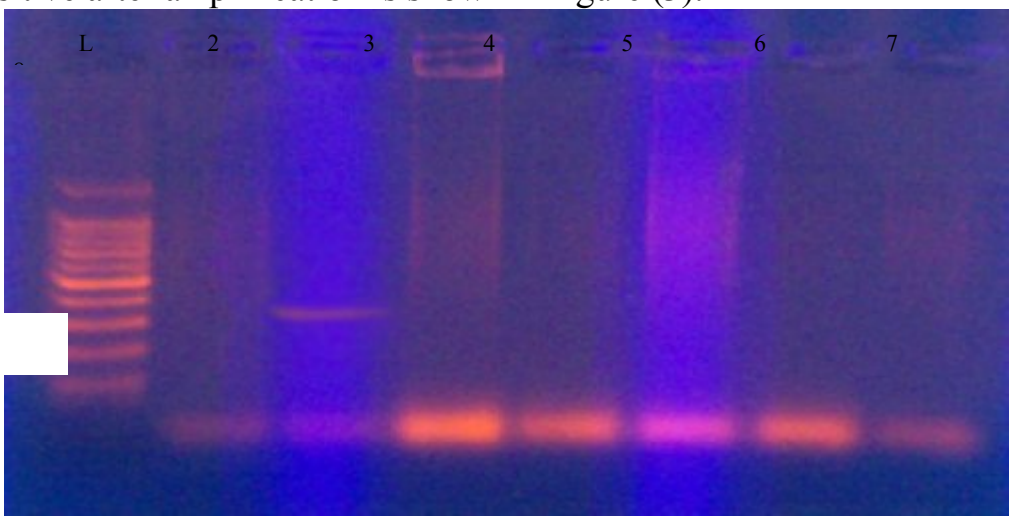
Figure(1):-Gel electrophoresis of PCR of *PAi* amplicon product. L: ladder; 2, 3, 4: no. of isolates obtained from urine; 5, 6, 7: no. of isolates obtained from stool; 8: no. of isolates obtained from rectal

papGI is absent from all isolates. While *papGII* is present in ten isolates includes urine (3 samples), vagina (3 samples), stool (2 samples), and rectal (2 samples)are positive after amplification is shown in figure (2).



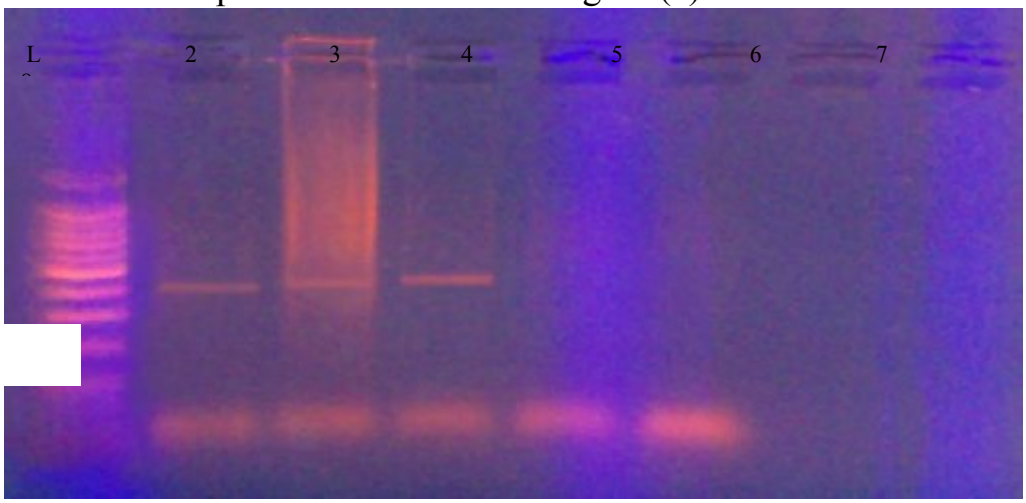
Figure(2):-Gel electrophoresis of PCR of *papGII* amplicon product. L: ladder; 2, 3, 4: no. of isolates obtained from urine; 5: no. of isolates obtained from stool; 6, 7, 8: no. of isolates obtained from rectal

In the same way, *papC* is present in one isolate which includes urine are positive after amplification is shown in figure (3).



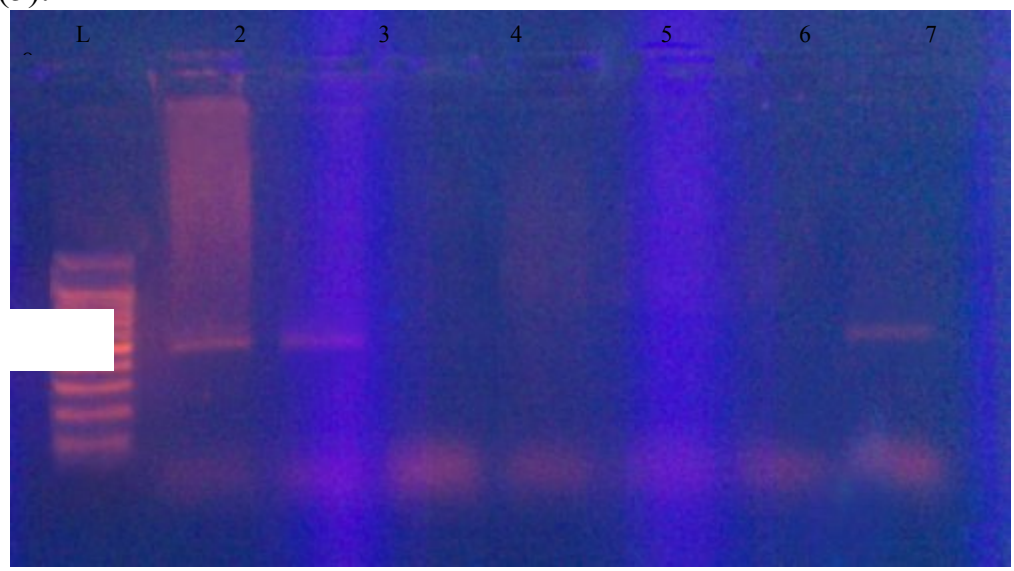
Figure(3):-Gel electrophoresis of PCR of *papC* amplicon product. L: ladder; 2, 3: no. of isolates obtained from urine; 4, 5, 6: no. of isolates obtained from stool; 7, 8: no. of isolates obtained from vagina

Also, other types of adhesin *sfa* is present in only three isolates, includes urine (1 sample), vagina (1 sample), and stool (1 sample) are positive after amplification is shown in figure (4).



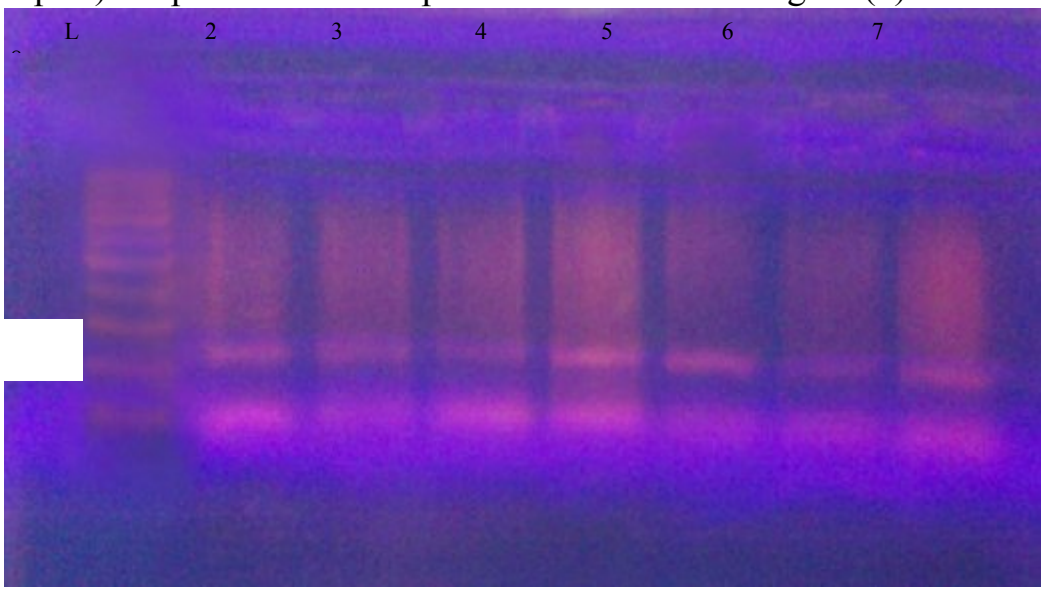
Figure(4):-Gel electrophoresis of PCR of *sfa* amplicon product. L: ladder; 2, 5: no. of isolates obtained from urine; 3, 6: no. of isolates obtained from stool; 4, 7: no. of isolates obtained from vagina; 8: no. of isolates obtained from rectal

The gene *hlyC/A* is present in five isolates, includes urine (3 samples) & vagina (2 samples) are positive after amplification is shown in figure (5).



Figure(5):-Gel electrophoresis of PCR of *hlyC/A* amplicon product. L: ladder; 2, 3, 4: no. of isolates obtained from urine; 5, 6, 7: no. of isolates obtained from rectal; 8: no. of isolates obtained from vagina

Aerobactein gene *iucC* is present in eight isolates, which includes urine (1 sample), vagina (1 sample), stool (3 samples), & rectal (3 samples) are positive after amplification is shown in figure (6).



Figure(6):-Gel electrophoresis of PCR of *iucC* amplicon product. L: ladder; 2, 3, 4: no. of isolates obtained from rectal; 5, 6, 7: no. of isolates obtained from stool; 8: no. of isolate obtained from vagina

Discussion

Pathogenic isolates of *E. coli* are known to carry large chromosomal regions required for virulence, which are termed PAIs. In this study, 12 *E. coli* isolates from clinical samples were characterized using PCR with primers specific for PAIs genes includes: *PAi, papG(I, II), papC, sfa, hlyC/A, and iucC*.

It was found the *PAi* is present in only one isolates (urine). This result indicate that this isolate contains all virulence genes are included in this study, and for that this isolate may contain true pathogenicity islands. The high prevalence of the PAI marker observed in the present study is consistent with previous study by Johnson and Stell (19), who have been reported that this marker may be found in clinical isolates, so PAIs also may contribute to urovirulence and may potentially serve as targets for interventions.

Many adhesive factors are seen in *E. coli* these play a major role in the colonization of *E. coli* in different tissues in human body. *papGI* is absent in all isolates. This result is agreement with Tseng *et al.,* (20) who have observed that *papGI* is uncommon and rarely found in human. While *papGII* gene is located in pathogenicity islands was found in ten isolates. Our results highlight higher frequency of *papGII* compared with the rest of the genes, and this may indicate a crucial role of the virulence genes associated with *E. coli*.

Marss *et al.,* (21), found that *papGII* is present as high prevalence in those obtained from UTI (59.3%), than fecal isolates (34.2%). These data suggest that the prevalence of *papGII* varies directly with severity of UTI. study of healthy adults has found up to 50% of *E. coli* strains carry *papGII* (22). However, these results vary widely and may be attributable to geographical and lifestyle differences.

Furthermore, *papC* is detected in only one isolate. This study is in agreement with Ananias and Yano (23) who found that nearly 5% of strains were positive for *papC* and other were negative, suggesting that other adhesive gene was present or *papC* may be deleted. Also, Daoud *et al.,* (24), have isolated twenty isolates from various clinical material (urine, vagina, and rectal swab), and it has been found that three isolates gave *papC* and the others did not produce it.

sfa located within PAIs was observed in only three isolates The distribution of the sfa found among the isolates studied was higher than previous studied by Santo *et al.,* (25) who reported that only one isolate of *E. coli* bear this gene. Also, the S fimbriae did not appear to act as essential VF because the total prevalence of *papGII* was much higher and the results are consistent with several other studies by Ananias and Yano (23); Johnson and Stell (19).

Because iron acquisition is essential to the survival of invasive strains of *E. coli*, the frequency of the two potential iron acquisition systems, hemolysin and aerobactin production, were compared in *E. coli* isolated from clinical samples.

hlyC/A associated with pathogenicity islands was present in five isolates This results is lower than obtain by Escorbar-Páramo *et al.,* (26) who found that 11 isolates from 22 isolates contain this gene. *Hly* operon can be present within PAIs, this gene as representative of ExPEC

virulence factors to reconstruct its evolutionary history by sequencing *hlyC/A*. Sequencing of *hlyC/A* revealed that some strains exhibit two genes, as previous reported (27). The same PAIs harboring unique *hlyC/A* sequence can be found in diverged strains. Also, it was found that the five isolates was positive to *hlyC/A* had the ability the ability to produce extracellular hemolysin.

Similarly, aerobactein gene *iucC* located within PAIs was found in eight isolates, This result is agreement with Birošová *et al.,* (11), who have been shown that that aerobactin were detected more frequently among rectal isolates (76%) than among urine (53%) or vagina isolates (49%). This finding suggests that iucC is advantageous for the survival of *E. coli* strains in the gut. The precise role of aerobactin in intestinal carriage of uropathogenic *E. coli* strains or those associated with diarrhea in children remain to be determined.

Also, this result show that one isolate of *E. coli* (urine sample) contain the gene *hlyC/A* and *iucC*, this indicating a synergistic action of the hemolysin with the aerobactin receptor , other explanation that this two gene located on the same operon of PAIs.

These results indicate that each steps in the infection process can be mediated by a number of alternative virulence factors and each strain may have a unique combination of these factors. This assortment of virulence genes is made feasible by the variety of genetic factors contributing the genome plasticity, such as plasmid, phages, and transposon elements.

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