Detection of the, hlyA, fimH PgaD gene in Escherichia coli isolated from different sources in Kirkuk city

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Abstract

This study was directed for the period from November 2021 to April 2022, where (155) clinical samples were collected from diverse patients who suffer from various pathological injuries, and they included burn injuries (25)specimen, and wounds (25) specimen, and urinary tract infections, urine specimen (50) specimen, and sputum specimen (55) specimen , This study came to discover some genes of virulence elements for bacteria Escherichia coli, Bacterial isolates analyzed using traditional methods, and then their identification was confirmed by molecular detection of hlyA, fimH, and PgaD genes by polymerase chain reaction (PCR) technology, as (42) clinical isolates belonging to the type E. coli were obtained, and the results showed that (39) isolates with a rate of (92.86%) possessed PgaD genes . (29) isolates (69.05%) possessed the hlyA gene and (37) isolates (88.10%) possessed the fimH gene. The study showed that all E. coli isolates isolated from wounds and sputum contained the PgaD gene with a percentage of (100%), and the isolates of E. coli isolated from the urine (18) isolates and from burns (6) isolates contained the PgaD gene by (90%) and (85.7%), respectively. E. coli isolates isolated from the urine (15) isolates (75%) and from burns (5) isolates (71.43%) and from sputum (3) isolates (60%), and from wounds (6) isolates (60%) contained the hlyA gene, and all isolates of E. coli isolated from sputum (5) isolates (100%) contains the FimH gene, and 19 isolates (95%) isolated from the urine, and (6) isolates (85.71%(The isolates from burns and (7) isolates (70%) from wounds contain the Fim gene.

Keywords: *hlyA, fimH, PgaD, Escherichia coli.*

INTRODUCTION

E. coli is characterized by its production of the enzyme Haemolysin, which plays an significant role in its pathogenicity(1). As this enzyme works to form holes in the cell wall and dissolve red blood cells, and this aids to release important nutrients such as iron, which has a role in the growth and spread of bacterial cells, and E. coli bacteria can produce several types of hemolysin, the most important of which is alpha-hemolysin(2), which is a protein secreted outside the cell And it forms holes in the cell wall, and this type is called α-hemolysis, and the other type is beta-hemolysin, which is a protein associated with the cell that analyzes the bacterial cell membranes, and this type is called β-hemolysis. This enzyme is secreted by strains of E. coli bacteria, especially strains that infect the urinary system, and some of its strains present in the intestine can produce this enzyme and it is considered one of the important virulence factors for this bacteria(3). There are four genes that encode this enzyme (hlyA, hlyB, hlyC, hlyD). It is carried on the plasmid or chromosome that is acquired through horizontal gene transfer(4). bacteria E. coli are also distinguished by their multidrug

resistance (MDR), as they are characterized by their high resistance to antibiotics as a result of their possession of resistance enzymes such as β-lactamases that confer Resistance to beta-lactams, , and enzymes that confer resistance to aminoglycosides, , and to quinolones. These bacteria also have other mechanisms that confer resistance to antibiotics, such as changing the permeability of the cell membrane, changing the target site, and inhibiting protein synthesis(5).

Materials and methods:

Hemolysin production test

Bacterial isolates were planted on dishes containing blood agar medium, and the dishes were incubated at 37 °C for 24 hours, then the results were read by observing the type of decomposition (alpha, beta, and gamma).

α-hemolysis : partial lysis around the peri-colonial area

β-hemolysis : Total decomposition around the peri-colonial area in the form of a transparent halo.

ɣ-hemolysis: no hemolysis was observed around. the colonies.(6)

DNA extraction

Bacterial isolates were grown on Brian heart infusion broth culture medium and incubated at 37 °C for 24 hours, and genomic DNA was extracted from the bacterial growth according to the protocol of the Presto™ Mini gDNA Bacteria kit.(7)

Electrophoresis process:

PCR results were electrophoresed by 1% agarose gel with 3 µl of Safe Red dye in SB X1 solution using a DNA ladder 100-1500 bp (Bioneer, Korea) at 90 V for 80 min, after the end of migration the agarose gel was examined Using an ultraviolet light source using a UV-Transilluminator at a wavelength of 260 nm to investigate the DNA, and the results were photographed using a gel viewer.

Polymerase chain reaction (PCR)

Prepare this reaction mix using a PCR PreMix® AccuPower kit according to the company's instructions

manufactured as follows:

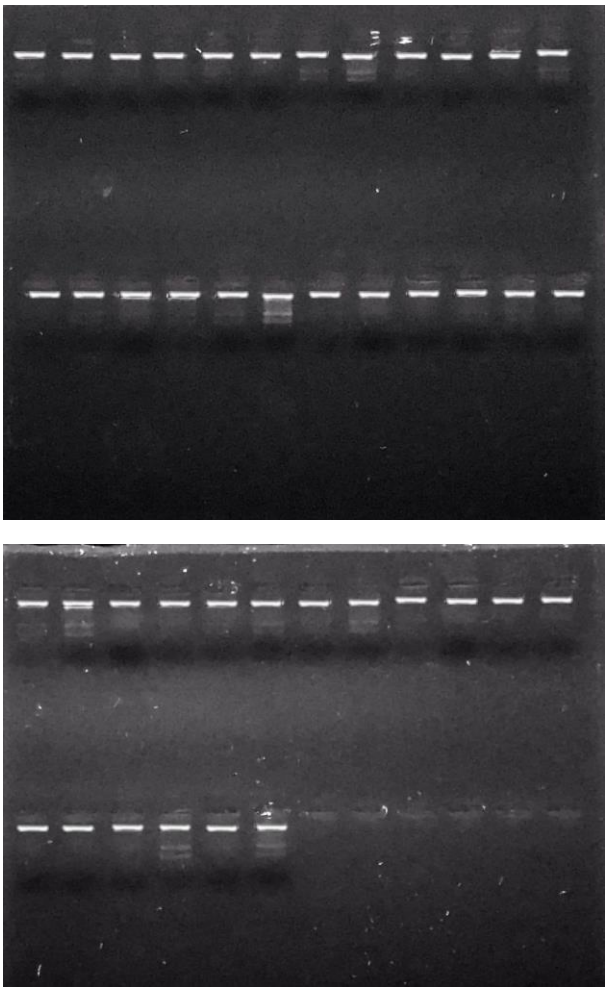
1- The polymerase chain reaction (PCR) mixture was prepared in the PCR tubes that were equipped with the kit and contained on PCR PreMix components prepared by Bioneer (Korea), 3 µl of template DNA extract, 1 µl of F-Primer, 1 µl of R-Primer and 15 µl of nucleic acid-free distilled water prepared by Bioneer (Korea). Then the contents of the PCR tubes were mixed well.

2- The tubes were transferred to a thermocycler for the polymerase chain reaction for the purpose of conducting DNA amplification operations according to the optimal thermal conditions for the cycles represented in the processes of separating the DNA denaturation, associating the primers with the separated strand, annealing and lengthening the Extension DNA chain.

Results and discussion:

The DNA of the bacterial isolates E. coli, which was diagnosed from different disease cases, was extracted by (42) isolates, and the extraction results showed clear bands for all the studied isolateءفقs, and the integrity of the extract was confirmed after it was transferred on an agarose gel with a concentration of (1%).

Figure: 1 DNA migration of (42) E. coli isolates



Molecular investigation of virulence factors genes hlyA, fimH and PgaD by PCR polymerase chain reaction

A number of genes responsible for the virulence factors of the bacteria E. coli were investigated, as the presence of these genes is an important factor in their virulence, as well as in adaptation to different environmental conditions, where three primers (hlyA, fimH, and PgaD) were used, which encode the virulence genes of the bacteria. .

Molecular detection of the PgaD gene using PCR technology

The results of the molecular detection of the PgaD gene, which has a size of 340 base pairs, using PCR technology and using a thermo cycler, indicated that 39 bacterial isolates possessed the PgaD gene. By comparing the replication bands with those of the DNA ladder, it was found that the resulting bands had a molecular weight of 340 pairs / Base as shown in Figure (2)

The study showed that all E. coli isolates isolated from wounds and sputum contained 100% of the PgaD gene, and that 18 E. coli isolates isolated from urine and 6 from burns contained PgaD gene by 90%, 85.7%, respectively.

Figure 2: Molecular detection of the PgaD gene in E. coli using the PCR technique

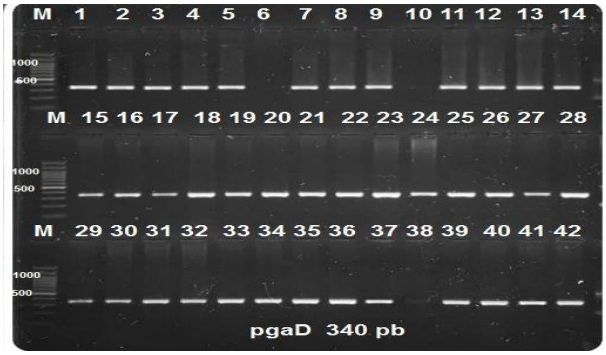


Table 1: Detection of the PgaD gene in E. coli isolated from clinical samples

		The number of isolates that do not have the gene		The number of isolates that have the gene			
%	No	%	No.	%	No.		
100	20	10	2	90	18	20	Urine
100	5	0	0	100	5	5	Sputum

100	10	0	0	100	10	10	Wounds
100	7	14.285	1	85.714	6	7	Burns
100	42	7.142	3	92.857	39	42	Total
		ns Chi-Square = 4.889 P-Value = 0.033		**** Chi-Square = 14.325 P-Value = 0.002**			

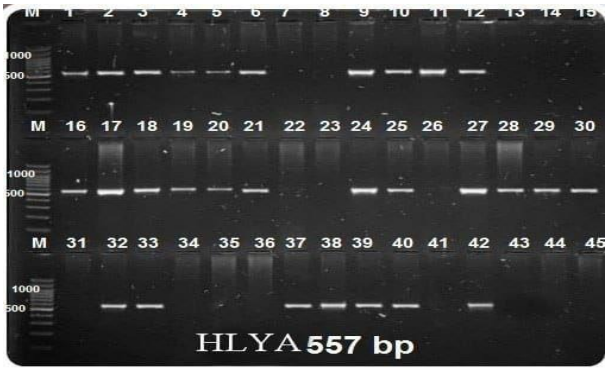
The statistical results in Table (1) showed that there was a significant difference in the number of E. coli isolates that possess the PgaD gene at a probability level (P-value 0.002).

PgaD may assist the glycosyltransferase PgaC in the polymerization of PGA (8). E. coli biofilm formation is closely related to healththreatening infection and is also a mechanism of antimicrobial resistance (9). To control the biofilm formation of E. coli, studies on natural phenolic acid has increased in recent years(10). In the present study, our results showed In E. coli, the synthesis and secretion of PGA are regulated by the pga operon which encodes a series of porins PgaD. PGA is considered to be the key polysaccharide in the biofilm formation which catalyzes the formation of irreversible adhesion between E. coli cells, thus ensuring the formation and maturation of a biofilm (11). In other words, the biofilm will not continue to form if there is no adhesion occurring in this step. We found that E. coli growth was significantly affected. Previous studies have shown that pga operon and polysaccharide synthesis is induced by NaCl, ethanol, and glucose, but that only glucose augments biofilm formation (12)

Molecular detection of hlyA gene using PCR technology

The results of the molecular detection of the hlyA gene, whose size is (557) base pairs, using the PCR technique and using a thermocycler, showed that (15) E. coli isolates isolated from the urine 75%, (5) isolates from burns71.43% , (3)isolates from sputum60%, and 60% from wounds, were 6 isolates, It contains the hlyA gene, and by comparison between the replication bundles and those of the DNA ladder, it was found that the resulting bundles had a molecular weight of 557 base pairs, as shown in Figure (3) and Table (2).

Figure: 3: Molecular detection of the hlyA gene in E. coli using the PCR technique



In current study was hlyA gene that encodes for hemolysin exotoxins (13), . HlyA is a type of toxin that helps bacteria enter the bloodstream through E.coli colonies where these toxins are produced for the decomposition of erythrocyte and the nucleated host cell. This process helps break down the immune system cells (14). HlyA is one of the important virulence factors possessed by E. coli bacteria. This gene has an important role in skin and urinary tract infections in humans caused by pathogenic E. coli bacteria. It is a lipid protein that degrades red blood cells of the host and promotes bacterial secretion of hemolysin and increases

the ability of bacteria to reach To the cells of the affected area (15).

Table 2: Detection of the hlyA gene in E. coli isolated from clinical samples

Total		*hlyA* gene				Source of isolates
		The number of isolates that do not have the gene		The number of isolates that have the gene		
%	No.	%	No.	%	No.	
100	20	25	5	75	15	**Urine**
100	5	40	2	60	3	**Sputum**
100	10	40	4	60	6	**Wounds**
100	7	28.57	2	71.43	5	**Burns**
100	42	30.95	13	69.05	29	**Sum**
		Ns Chi-Square = 2.769 P-Value = 0.429		**** Chi-Square = 15.586 P-Value = 0.001**		

The statistical results in Table (2) showed that there was a significant difference in the number of E. coli isolates that possess the hlyA gene at a probability level P-value (0.001).

Molecular detection of the FimH gene using PCR technology

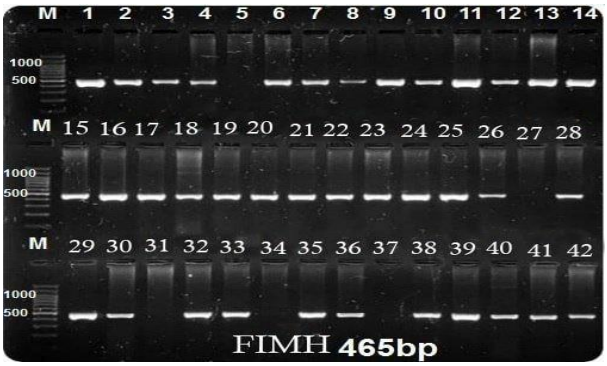
The results of the molecular detection of the FimH gene, whose size is (465) base pairs, using the PCR technique and using a thermo cycler, showed that all E. coli isolates isolated from sputum (100%) contain the FimH gene and E. coli isolates isolated from urine.(95 %) and (85.71 %) isolated from burns and (70 %) from wounds contain FimH gene. Comparing the replicated bundles with the bundles belonging to the DNA ladder, it was found that the resulting bundles had a molecular RNA (465) base pairs as shown in Figure.

Table 3 Detection of the FimH gene in E. coli isolated from clinical samples

Total		*FimH*				Source of isolates
		The number of isolates that do not have the gene		The number of isolates that have the gene		
%	No.	%	No.	%	No.	
100	**20**	**5**	**1**	**95**	**19**	**Urine**
100	**5**	**0**	**0**	**100**	**5**	**Sputum**
100	**10**	**30**	**3**	**70**	**7**	**Wounds**
100	**7**	**14.29**	**1**	**85.71**	**6**	**Burns**
100	**42**	**11.90**	**5**	**88.10**	**37**	**Total**
		Ns		******		

		Chi-Square = 5.067 P-Value = 0.167	**Chi-Square = 18.559 P-Value = 0.0004**	

Figure 4: Molecular detection of FimH gene in E. coli using the PCR technique



fimH encodes fimbriae H which bind with uroepithelial protein α-D-mannosylated, stabilizing adhesion to the host's uroepithelial under extreme pressure which in turn could potentially lead to urosepsis while pap encodes P fimbriae which is associated with cystitis and pyelonephritis cause it binds to α-D-galactopyronosol-(1-4)-β-D-galactopyronoside-containing receptor found on upper urinary tract(16).. The wide variety of virulence factors encoded by associated genes present in E. coli are acquired via DNA transfer to plasmid, transposon, bacteriophage, and pathogenicity island (PAI) help it in overcoming host's defenses and causing UTIs. Type 1 fimbriae in E. coli is encoded by the chromosomal fimH operon (17). FimH mediates E. coli binding to mannose-containing glycoprotein receptors-uroplakins, which are located on the luminal surface of the bladder epithelial cells (18). Entertainingly, the FimH adhesin mediates both bacterial adherence to and invasion of host cells and contributes to the development of intracellular bacterial biofilms by UPEC (19).

Conclusions:

The PCR technique demonstrated a high speed of sensitivity and specificity,and it is a fast and efficient method for detection the presence or absence of virulence factors genes in the samples under study. most of the E. coli isolates under study possess genes encoding virulence factors FimH, PgaD, , hlyA the results of the study showed that (14) isolates of E. coli has the ability to produced haemolysis on blood agar.

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