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The Wide Spread of the Gene Haeomolysin (Hly) and The Adhesion Factor (Sfa) in The E.coli Isolated From UTI

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Abstract

The current study was conducted for the period from the beginning of the month of 2016 until the end of March 2017. 100 samples were collected from patients with UTIs, to Samarra General Hospital and the laboratories of the districts and surrounding areas of the judiciary and of both sexes and different ages, including the isolation and diagnosis of Eoli and investigate the extent of the spread of some of the factors of virility in selected isolates. Forty isolates of the developing isolates were isolated after culturing them on the different media through microscopic, culturing and biochemical characteristics. The results of the diagnosis showed that 40 isolates, 40%, belonged to E. coli, while the rest were from other species.

%9100 ,%97.5 ,(%95.5)Respectively, followed by Ceftazidime, 92.5%, Gentamicin (75%) and Ciprofloxacin (72.5%). For the antagonists (Norfloxacin, Nitrofurantin), the lowest resistance was found (32.5% and 50%) respectively. The production of these isolates was investigated by transplanting them to the blood acacia where 30 isolated isolates were isolated (Hemolytic-ß), 7 hemoglobin (α-hemolytic) and 10 isolates (non hemolytic).

Thirty isolates were selected based on their ability to produce most laboratory-tested virulence agents and their resistance to many antibiotics to assess the spread of hly, SFa genes using specialized primers and using PCR technique.

PCR results showed the presence of 13 mg of isolates in 43 isolates, 43.5% of Ecoli isolates under study, and the presence of the gene encoding the SFA was detected. The results showed that the gene was present in only 4 isolated isolates of the bacteria is 13.3%.

INTRODUCTION:

Ecoli is an opportunistic pathogens that rarely cause infection in healthy individuals but can cause infections in Immuno Compromised hosts. The mechanism that is able to cause E.coli to cause infections Is the possession of many factors of virility and to be able to make the infection should enter enough to overcome the defenses of the host, and the pathogenicity of these bacteria in the bacterial adhesion and colonization, and helps the adhesion factors colonization factors antigen E.coli bacteria in the adhesion of these bacteria and colonization of host tissues About (1), the bacterial determinants of virulence factors mediate all stages of the disease and are therefore responsible for the occurrence of the infection. For example, the possession of E. coli for the fermentation agent of the hemolycin increases the pathogenic effects of the germ. Ecoli is part of the natural plant in the intestines of healthy individuals, but there are strains that can cause diseases in the digestive system as well as urinary tract infections. Researchers (3) (4) referred to the role of E. coli in inflammation of the mesenteric peritonitis, septicemia, meningitis, and bile duct inflammation.

E.coli is one of the most important intestinal species, because it is normal in different areas of the body. In the ear, burns may be found in the intestines. E.coli bacteria lead to lower parts of the urinary tract and bladder. E.coli has many virulent agents that cause disease, and also affects the lower parts of the respiratory system, especially after surgery (5). E. coli can also be found in many It is possible to control the spread of E. coli through the correct isolation of patients and to observe the conditions of cleanliness, catheterization and intercourse and periodic emptying of the bladder (6).

THE MATERIALS AND SEPARATION METHODS

Samples Collection:

A total of 100 samples of Urine samples were collected for patients of both sexes with ages ranging from 4-85 years of patients of Samarra General Hospital and external laboratories suffering from the symptoms of urinary tract infections, namely heartburn and urinary incontinence. The samples were collected in sterile plastic bottles. (Clean-Catch-Midstream Urine) after washing the genital area with soap and water to avoid contamination with the natural vegetation found in this area (7) .

Samples Agriculture

Samples were planted on the following agricultural communities:

1-Mac Conkey Agar Medium

2-Blood Agar Medium

The bacteria were planted on this medium for the purpose of detecting their ability to produce Haemolycin and decomposition of red blood cells and incubating the dishes at 37 ° C for 24 hours. The appearance of a transparent halo around the colonies indicates the ability of E.coli bacteria to analyze blood.

3 -Eosin- blue methylen

The bacteria were planted at this age (18-24) hours and incubated at 37 ° C for 24 hours, with growing colonies appearing in bright metallic green.

3- Diagnosis of bacterial isolates

Cultural Characteristics

The characteristics of individual colonies were studied on the blood agar medium and the Eosin Metheline Blue (EMB) medium and its fermentation of lactose sugar found in the center of MacConkey's agar.

Table 1

Gene and DN region amplified	Primer	Primer sequence(5-3)	Size(bp) product
hly	F	AACAAGGATAAGCACTGTTCTGGCT	1.177(bp)
	R	ACCATATAAGCGGTCATTCCCGTCA	
Sfa	F	CTCCGGAGAACTGGGTGCATGTTAC	410(bp)
	R	CATCAAGCTGTTTGTTCGTCCGCCG	

Table 2

(%) Percentage	Number of resistant isolates	Antibiotic Types
97.5	39	Ampcillin
75	30	Gentamicin
95	48	Amikacin
72.5	29	Ciprofloxacin
92.5	37	Ceftazidim
82.5	33	Cefotaxim
100	40	Trimethoprim
50	20	Norfloxacin
32.5	13	Nitrofurantin

Microscopic Examination

Methods used by (9) were used, where thin swabs from the colonies were observed to observe their chromatin pigmentation and to identify the cell forms. Hanging Drop Methods were also detected using a slide designed for this purpose.

Biochemical Tests

The following tests were performed

Genomic DNA isolation

Several genome-derived genomic DNA extracts and purification were used by Promega (USA) to isolate genomic DNA from identified bacterial isolates.

Specific primers used in PCR reactions

Specific prefixes were used to target the common genome sequence of genes.

(S-Family adhesion, hly), as indicated in (11). As shown in Table (1)

RESULTS AND DISCUSSIONS

Isolation and diagnosis of E. coli due to urinary tract infection

The total number of samples was 100 samples collected from people who were infected with UTIs in Samarra General Hospital for the period from the beginning of August 2015 until the end of March 2016. The results of isolation and diagnosis showed 50 isolates belonging to E. coli (50% The other isolates were of different genotypes. Ecoli bacteria were diagnosed based on morphological, enzymatic and chemo-enzymatic tests, which appeared as short negative chromosomes. All isolates were fermented to lactose on the Maconkey and in circular colonies with convex and pink edges, and the center of the Eocene was blue green with metallic green luster. All isolates were produced by the enzyme Catalysis and Indole because they had the ability to split Tryptophan and non-oxidase.

Resistance to the isolation of E.coli antibiotics

The multiple resistance of bacterial isolates and more than one antibiotic is one of the major and serious medical problems because it is difficult to test the appropriate treatment for the patient. One of the main reasons for the emergence of multiple resistance is the indiscriminate use of antibiotics without relying on a sensitivity test, which increases the chances of adaptation Bacteria and their resistance to antibiotics used in treatment (12) Bacteria may appear resistant to many antibiotics, as a result of the transfer of the R-Factor resistance factor, which may be responsible for increased resistance, especially in patients who have long period in hospitals.

The sensitivity of 50 isolates to 10 types of antibiotics was given. As indicated in Table 4.3, the following results were obtained: Trimethoprim, Ampcillin, Amikacin (95%, 97.5%, 100%) respectively, followed by Ceftazidime antagonist 92.5% (80.5%) and Ciprofloxacin (72.5%) compared to Norfloxacine (Nitrofurantine). The lowest resistance was (50% and 32.5%), respectively, with resistance to Cefotaxime (82.5%). (13) In a study on isolated colon bacteria from hospitals in Saudi Arabia, increased resistance to antibiotics, Ampicillin resistance (63%). This clearly means high resistance, as we found in our present study which was higher than this rate of 97.5%. The result is that the bacteria have resistance against the antibiotic Ampicillin, as the results agree with the addition of (14) and (15)). These bacteria were resistant to (89.9%) and (100%) respectively, and supported (15) resistance of these bacteria to this antibody and by (64%), and (16) that the resistance of these bacteria to ampicillin was 63%. It is known that colonic bacteria have resistance against Ciprofloxacine. In this study, the resistance rate was 72.5%. The reason for the high resistance to this antibody is due to the presence of these genes, a gene responsible for the resistance, as well as the possibility of mutations leading to alterations in the DNA gyrase, Mutations led to the production of efficient super-stream systems (Wanget al., 2004). Among the (17) resistance of these bacteria to the sporofloxacin was (32%). The researchers found that the resistance of the bacteria to the sporvloxacin (24%) was the same as in the present study. The results of the present study are not consistent with the results of the study. [19]

Which found that the resistance of these bacteria to this antibody was (15%), and our results differed with what was obtained (13) about the resistance of these bacteria to this antibody, which was (46%). This difference may be due to several reasons, In the hospital and are exposed to continuous daily treatment, the use of antibiotics may be suspect A daily cause for the emergence of isolates resistant to antibiotics, although the type of isolates and the different origin of the reasons that led to the difference in the proportion of resistance from the rest of the research in addition to the studied sample size. Most of the studies indicate resistance to Gram-negative bacteria, especially the intestinal family of antibiotics of various types, especially anti-beta-lactam antagonists (22). This increases the importance of these bacteria or infections. Which are often common in hospitals and in immunosuppressed patients where they are difficult to treat because these bacteria have different and different mechanisms in which they can resist these antibiotics. The genetic basis of antibiotic resistance is the result of the existence of genes responsible for this resistance and mobile On chromosome or plasmid and possess these genes are enzymes responsible for breaking down antibiotics and convert them to form an effective 23)). Gentamicin is a group of aminooglycosides that inhibit the manufacture of protein in the cell. E.coli is resistant to this antibody. Resistance is due to the presence of an inhibitory enzyme encoded by the plasmid, modulation of the 30 S ribosome, or reduced antibody entry into the bacterial cell (24).

Investigation of the genes (hly, SFa) in isolates under study

Testing of blood decomposition factor Haemolysin

The results showed that there was a molecular size of 1.177bp in comparison to the volume index 100bp in 13 isolates (43.3%) of the isolates of E.coli bacteria under study that encode the hlyysis factor. The prevalence of this gene was after the gene encoding the toxin necrosis factor in bacterial isolates Eocoli is under investigation and this confirms the importance of this factor for the infection and the tests conducted on blood acars showed that (30) isolates were able to analyze the blood (23) of which are fully hemolytic-β blood analysis and (7) (Hemolytic-α). The remaining 10 isolates were non-hemolytic. (7) isolates that were partially blood analyzer and (13) isolates of the blood analyzer in full and after the polymerization test showed that (7) isolates that were partially blood analyzer did not show positive results for this gene Except in two isolates (8.5). As shown in Figure (4-1), while (13) isolates that were fully analyzed for blood, the results showed that only (5) of these isolates (18,17,16,15,14) Were produced by this gene as shown in Figure (4-2), and (6) isolates (27,26,25,23,22,20) out of (10) non-analysis of blood on Akar Makonki was a producer of this gene and as shown in the picture (4-3) Itzadilil that it is not necessary to The results of the present study are relatively similar to the findings of 25 which found that 25% of the 48 isolates were the product of this gene and also came close to what was found (25) 26) who indicated that 67% of isolates of E. coli isolates from urinary tract infections possessed a hly gene. The results of the present study were similar to those of 27, Conducted on blood acars showed that four isolates out of 86 isolates were capable of blood analysis and hemolysis production but after the polymerization test the results showed that there were 9 isolates containing the virulence factor hly and this indicates that it is not necessary that the expression of blood decomposition as a virtual indicator On the presence or absence of algin, which encodes this status, as this enzyme works to dissolve red blood cells by forming holes Pore former in the membranes of cells targeted by its impact, which leads to the destruction of these cells to obtain the element iron and other nutrients by breaking red blood cells And edit within them from These materials by launching iron carriers Sidrophores.

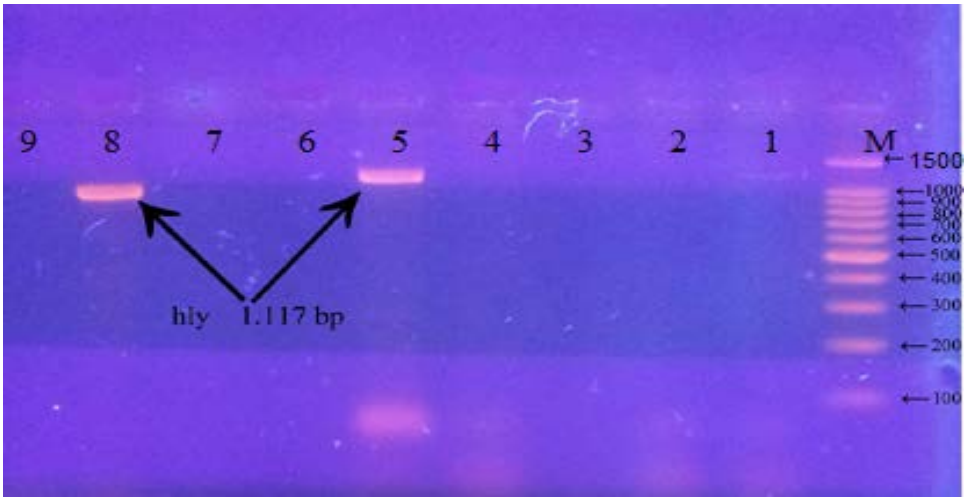

Figure (4-1) Electrophoresis of the PCR DNA product The isolates of E. coli (1-9) isolates from UTIs using specialized primers of hly hemolysin genes on 1.5% alkaloose gel and voltage difference (60) Two hours.

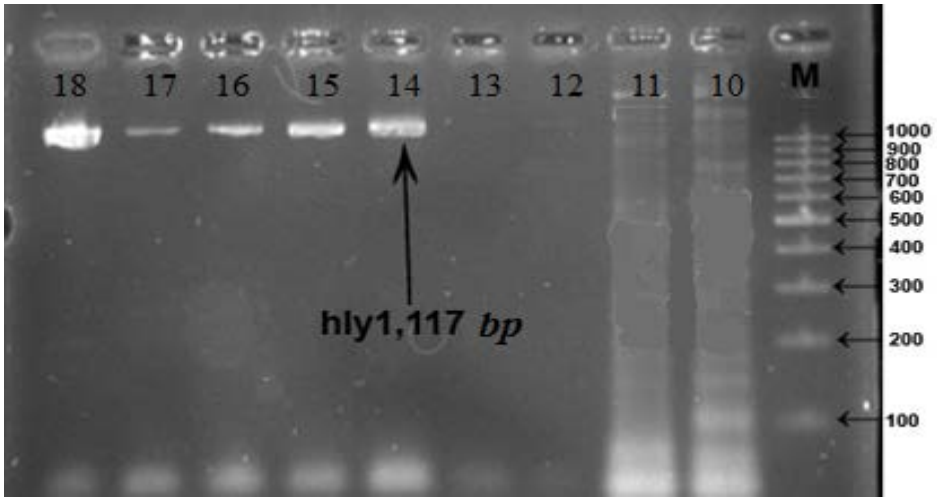

Figure (4-2) Electrophoresis of the PCR DNA product The isolates of E. coli (10-18) isolates from urinary tract infections using specialized primers of hly hemolysin genes on 1.5% alkaloose gel and voltage difference (60) Two hours.

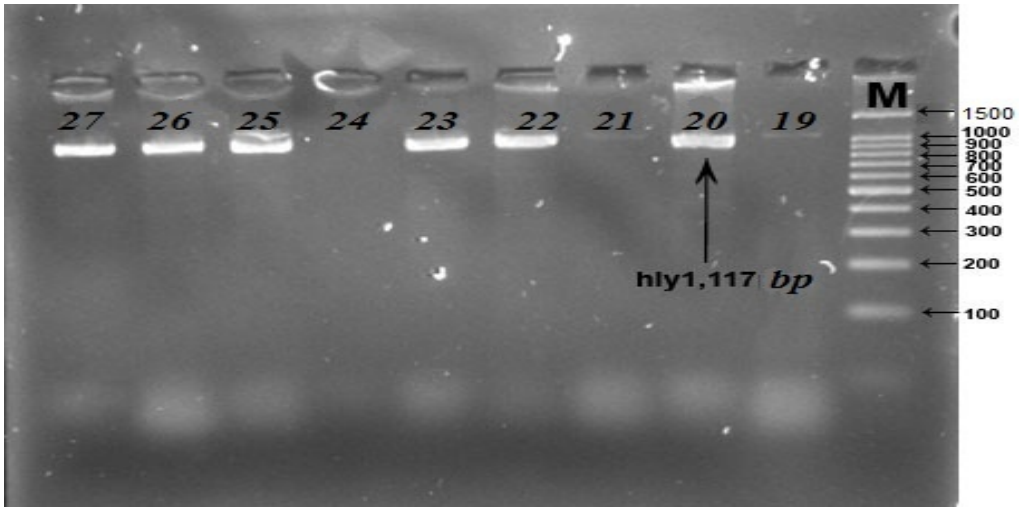

Figure (4-3) The electrical relay of the PCR DNA product isolates E.coli bacteria (19-27) isolates from UTIs using specialized primers of hly hemolysin genes on 1.5% alkaloose gel and voltage difference (60 V) Two hours.

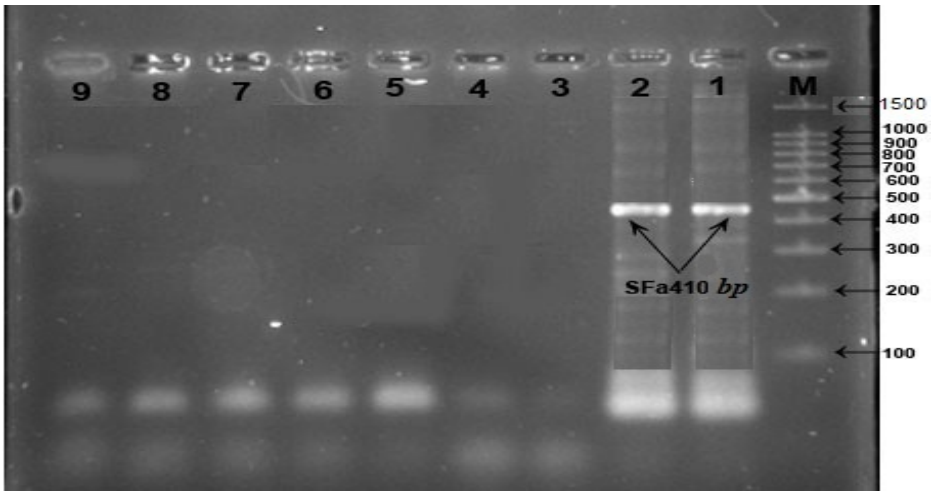

Figure (4-4) Electrophoresis of the PCR DNA product The isolates of E. coli (1-9) isolates from UTIs using specialized primers of SFa genes on 1.5% alkarose gel and voltage difference (60) Two hours.

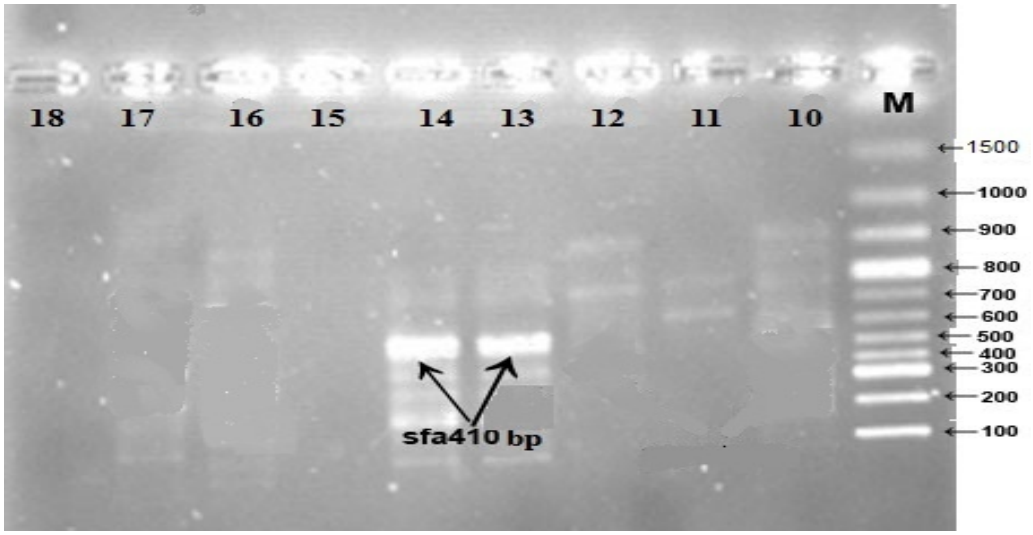

Figure (4-5) Electrophoresis of the PCR DNA product The isolates of E. coli (10-18) isolates from UTIs using specialized primers of SFa genes on 1.5% alkarose gel and voltage difference (60) Two hours.

Testing of S-family adhesion

The prevalence of SFA was investigated in 40 isolates of Ecoli isolates under study. The results of the electrical relay showed the presence of genetic bundles of the molecular size of bp410 compared to the volume guide bp100 in only 4 isolates (10%) of E.coli isolates under study (4-10).) Showing the existence of the beams in the isolates (1,2) and the image (4-11) revealed the existence of SFa encapsulation packages in the two isolates (13,14). The results of the present study were relatively similar to those found (28) Only 14.5% of E.coli isolates have this gene, which is also a small percentage. This may be due to the high prevalence of the gene (hly), as the results of our study Which is consistent with what the researchers reported 27 that when CNF-1 genes are present, the prevalence of SFa, PAP decreases. SFA is also a cause of intestinal infections and exogenous infections, including urinary tract infection and meningitis in neonates. E.coli bacteria help the bacteria to adhere to the surfaces of epithelial cells lining the urinary tract, where the researcher pointed out that the presence of genes (SFA, PAP) is linked to some, and observed in the isolates diagnosed from diseases of the urinary system and interpreted these researchers (28) Which indicated that these genes were responsible for production Two factors work together during the disease, where SFa works on adhering to the surfaces of the olfactory epithelial cells (18).

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