

# Isolation of Hemolysin - Producing Bacteria That Cause Infection in Patients with Urinary Tract Infections by Molecular Detection

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## ABSTRACT

The study included the collection of 200 urine samples from patients with urinary tract infection, and 130 isolates of *Escherichia coli* were diagnosed by relying on the principle of production to hemolysis enzyme. The isolates were collected from special laboratories in Iraq Baghdad, the research isolates the bacteria that cause urinary tract infections and produce hemolysis. In addition, the researches reach urinary tract infections and reveal their molecular characteristics and pathogenic factors associated with pathogenicity, hemolysis, and resistance to antibiotic control factors. Urinary Tract Infections (UTIs) are widespread, an important cause of morbidity, and resistance to antibiotic therapy is increasing. Women disproportionately suffer a UTI: 50 % of all women will have a UTI in their lifetime. Additionally, 20 - 40 % of those women who have an initial UTI will experience a recurrence with some suffering recurring recurrences with a serious deterioration in quality - of - life pain and discomfort, disruption of daily activities, increased health care costs, and fewer treatment options other than long - term antibiotic prophylaxis.

## KEYWORDS

*E coli*, UTI, Hemolysin, *S. aureus*

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## INTRODUCTION

The urinary system is responsible for storing and disposing of urine and its disposal of waste products excreted by the kidneys. The kidneys form urine by filtering dirt and excess water from the blood.<sup>1</sup> then its journey begins from the kidneys through two thin tubes called Ureters to reach the bladder. When the bladder is full, a person must urinate through the urethra. For the urinary system to function, it needs to cooperate with the lungs, skin, and intestines to maintain the chemical and water balance in the body.<sup>2,3</sup> Adults get rid of between 800 and 2,000 ml of daily fluids when consuming the recommended daily amount of fluids of up to 2 liters, according to the National Institutes of Health, but in some cases, in which certain types of drugs, such as those used to treat high blood pressure, are consumed, the amount of urine that this person produces is affected accordingly, and some types of drinks, that is, those that contain caffeine generate more urine because they are generating it.<sup>4-6</sup> Urinary tract infections are one health problem that afflicts a large proportion of the human community, estimated in the millions annually.<sup>7,8</sup> Urinary tract infections are also the most common cause of hospital infection, accounting for up to 35 % of hospital - acquired infections.<sup>9,10</sup> Females are more susceptible to infection than males because of their short urethra than males and its proximity to the hot and humid area surrounding the rectum teeming with microorganisms, where bacteria can reach the bladder more easily in females. A urinary tract infection affects any part of the urinary system: the kidneys, ureter, bladder, and urethra. Women are often more susceptible to urinary tract infection than men, and infection limited to the bladder is painful annoying. Severe consequences can occur if it moves to the kidneys and urinary tract infection occurs more often at a rate of 8: 1 among men and women. This means that for every eight women with a UTI, only one man has the disease.<sup>11-14</sup> Diabetes increases the incidence of urinary tract infection because it suppresses the immune system, which reduces the blood supply to most parts of the body. The high concentration of glucose in the urine of patients with high blood sugar causes pyelonephritis because this urine is a suitable medium for the growth and reproduction of bacteria.<sup>15,16</sup> There is a genetic predisposition to bladder infection in some families, in addition to other factors such as diabetes and lack of circumcision.<sup>17,18</sup> There are factors that cause complications, they are more ambiguous, including the predisposing causes such as anatomical and functional abnormalities, or those related to metabolic actions, as the infection of the urinary system in children is linked to vesicoureteral reflux (which is an abnormal reversion of urine from the bladder to the ureter and then to the kidneys and constipation. The possibility of a urinary tract infection increases in people with spinal cord injury due to the frequent use of catheters on the one hand and due to a defect in emptying on the other hand, and it is the most common cause of infection in this category. The discovery of antibiotics had a significant impact on decreasing the incidence of urinary tract infections and diarrhea. It was found that the excessive use of certain antibiotics randomly and without following a healthy regime leads to the emergence of strains of bacteria resistant to antibiotics. Usually, the resistance trait spreads exponentially with the increase in the use of these Antibiotics, which made the treatment of these diseases involve great difficulties, so it is necessary to choose the appropriate antibiotic for treatment,

not to be done randomly. Still, it depends on conducting drug sensitivity tests on isolated bacteria to find the appropriate antibiotic to eliminate them.<sup>19</sup> *E coli* bacteria is one of the important prokaryotic organisms used in scientific studies because it possesses an important group of characteristics such as its low generation time, ease of cultivation, and rapid growth to obtain important results on the genetic metabolic characteristics of living organisms cells. Therefore, bacterial cells were used as a biosensor to know the effect of gamma biology rays on the *E - coli* bacteria that reside in the intestines of humans and animals, and the effect of rays on living organisms is directly or indirectly on important molecules or organelles such as DNA and the cytoplasmic membrane.<sup>20</sup>

## MATERIALS AND METHODS

### Design Patient Sample

One hundred thirty isolates of Escherichia coli were diagnosed by relying on the principle of production to hemolysis enzyme. The isolates were collected from special laboratories in Iraq Baghdad.

### Research Design

The principle of molecular diagnosis was relied upon to investigate the hemolysis enzyme before and after mutation, in addition to knowing the full effect of radiation on the hlyA gene by relying on the presence of genetic mutations and the resistance of these isolates to antibiotics. Among adults between the ages of 20 and 50, urinary tract infections are about 50 times more common in women. In women in this age group, the majority of urinary tract infections are cystitis or pyelonephritis in men of the same age. If infection is suspected, curettage of the urethra is performed before urination to diagnose diseases, then urine is collected during spontaneous urination after hygienic treatment Urine examination is useful, but it does not provide definitive diagnostic information and defines pyuria as the presence of  $\geq 8$  leukocytes /  $\mu$ l of urine expelled, which is equivalent to 2 - 5 leukemias in the field of view on microscopic examination of urinary sediment. Most patients with candida infection have  $> 10$  WBCs /  $\mu$ l. The presence of non - pyuria bacteria, especially when different strains are detected, is usually caused by contamination during the collection process, and microarray has been observed in about 50 % of patients, but micro albuminuria is rare. Leukocyte casts, which may require special staining to distinguish them from renal tubular casts, only indicate an inflammatory response, they may be present in pyelonephritis, glomerulonephritis, and non - infectious tubular - interstitial nephritis.

### Study Period

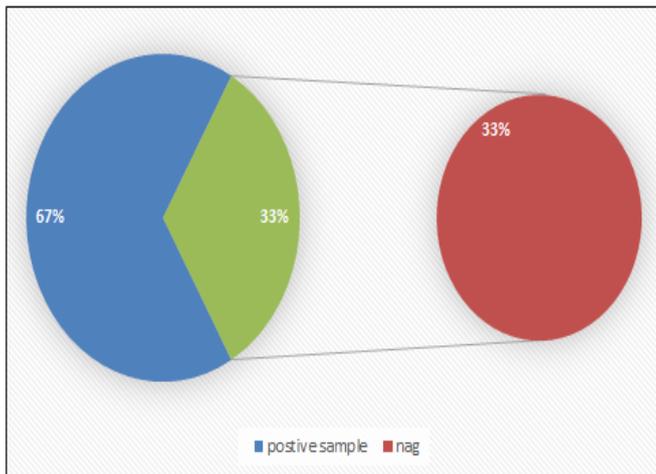
Demographic information and data were collected, and this study was conducted during more than one year period between 1 - 1 - 2019 to 6 - 6 - 2020.

### Aim of Research

The research aims to isolate the bacteria that cause urinary tract infections and produce hemolysis. In addition, the research reaches urinary tract infections and reveals their molecular characteristics and pathogenic factors associated with pathogenicity, hemolysis, and resistance to antibiotic control factors. Diagnostic test strips are often used where a positive reaction to nitrites in a freshly collected sample (bacterial growth in the container makes the result inaccurate if the sample is not examined immediately) is highly specific

for a urinary tract infection (Tables 1 - 8). The content of diagnostic information for samples contaminated with a large number of epithelial cells is unlikely. For inoculation, clean samples must be taken. Cultivation of a urine sample is more likely to reveal urinary tract infections. If samples are left at room temperature for longer than 2 hours, they can give false positive large numbers of colonies due to the continued multiplication of bacteria. Criteria for a positive culture result include isolate type one bacteria from a midstream urine sample, a clean urine sample, or a distilled urine sample (Figures 1 - 3).

**RESULTS**



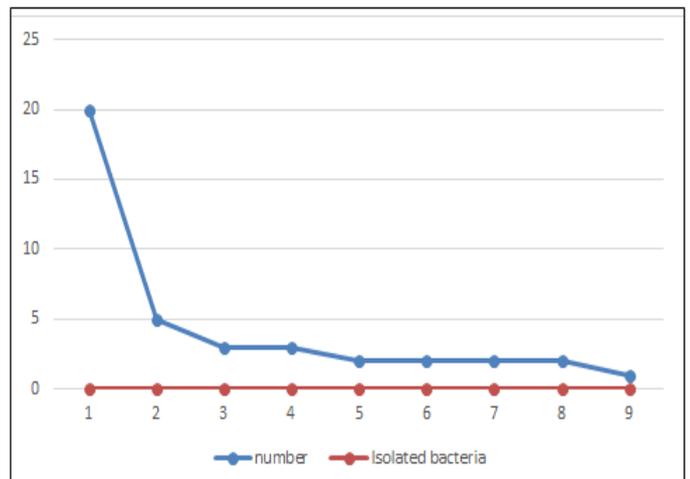
**Figure 1. Urine Samples.**

Staph epid	Entero bacter app	K. app	s. aureus	E coli	
+	-	-	+	-	G. stain
+	+	+	+	+	Catalase
-	-	-	-	-	Oxidase
- +	/	+	+-	/	Urease production
-	/	/	+	/	Goagulase
-	/	/	+	/	Manitole salt agar
S	/	/	+	A / A	Novobiocin sensitive
/	-	+	/	+	Indol
/	+	+	/	-	Citrate utilization
/	-	-	/	+	Methyl red
/	+	+	/	-	Voges Proskauer

**Table 1. Biochemical Tests to Diagnose Bacteria.**

Isolated bacteria	Number
<i>E. coli</i>	40
<i>Staph aureus</i>	25
<i>kleibSELLA</i>	24
<i>Entrobacter spp</i>	20
<i>Staph epid</i>	10
<i>Staph haemolyticus</i>	4
<i>P mirabilis</i>	3
<i>providentia spp</i>	2
<i>P. Vulgaris</i>	2

**Table 2. Bacteria Isolated from Urinary Tract Infections.**



**Figure 2. Disturbance Isolated Bacteria.**

T	M	F
Total	100	100
Positive	70	60

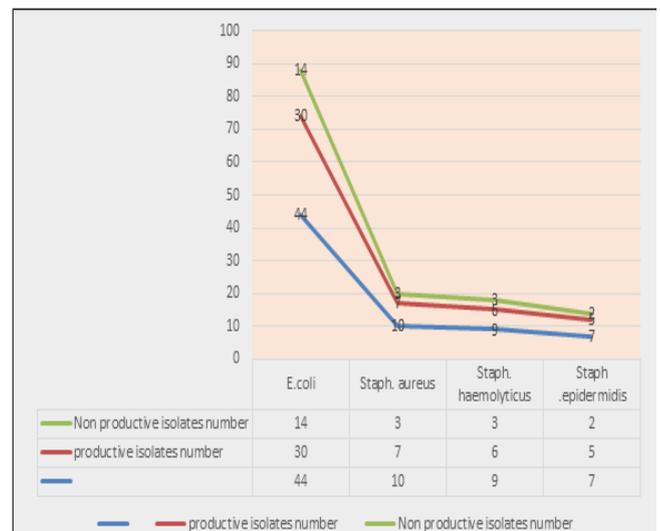
**Table 3. Patient Depend on Gender.**

	P-value
Total	0.003
positive	0.01

**Table 4. P-value.**

T	N
lysate bacteria isolates	40
Non degrading bacteria	90

**Table 5. Results Haemolysin of Detection.**



**Figure 3. Numbers and Percentages of Bacterial Isolates Depend on Isolated Hemolysin.**

<b>Bacterial isolated total</b>	<b>0</b>
<i>E. coli</i>	0
<i>Staph aureus</i>	0.1
<i>Staph haemolyticus</i>	0
<i>Staph epidermidis</i>	0
<b>Table 6. P - Value between Non - Productive Isolates and Productive.</b>	

Bacteria isolates	N total	production of hemolysis	production of hemolysis	production of hemolysin	production of hemolysin
		A	B	AB	O
<i>E coli</i>	15	6	15	23	13
<i>Staph aureus</i>	10	5	8	10	9
<i>Staph epidermidis</i>	8	4	7	5	3
<i>Staph. haemolyticus</i>	7	3	6	2	2
<b>Table 7. Number of Bacterial Isolates Analysed For Human Blood Species.</b>					

	A	B	AB	O
<i>E coli</i>	0.93	0	0	0
<i>Staph aureus</i>	0.08	0	0	0.2
<i>Staph epidermidis</i>	0.01	0	0.1	0
<i>Staph haemolyticus</i>	0	0	0	0.1
<b>Table 8. P - Value of Number of Bacterial Isolates.</b>				

**DISCUSSION**

The bacteria causing urinary tract infections were diagnosed after careful study and purified with approval. On the culture characteristics of the colony on solid media, the microscopic characteristics under microscopy were also relied upon to determine the shapes, arrangement, and arrangement of the isolated Gram - stained bacterial cells, *E - coli* is in the form of short non - forming Gram - negative sticks, as is the case for Staphylococcus. Microscopic examination of the stained cells was scant; it's a clustered arrangement of Gram - positive coccia of Gram - staining cells. There are many different bacteria that can cause a Urinary Tract Infection (UTI), but the most common type is *E coli*, which normally live in the intestines and can infect the urinary tract when stool is exposed to the urethra. Sometimes other types of bacteria are the cause. The number of *E coli* bacteria was 40. The most common type of Urinary Tract Infection (UTI) is a lower urinary tract infection, in which bacteria infect the urethra and bladder. There are highly virulent strains of the bacteria that, if left untreated, can spread to the ureters and kidneys, causing an upper urinary tract infection. Then the symptoms get significantly worse, causing, for example, back pain, nausea, and fever. Kidney inflammation is serious and can damage or lead to kidney failure. If left untreated, the infection can enter the bloodstream, requiring intensive care. Aurous. Staph is also a common cause of infections among younger women, but sometimes other bacteria are the cause. Women are more at risk than men, mainly because of their female anatomy. The urethra in women is relatively short and is also located near the anus, where bacteria can penetrate into the urinary tract. The number of lysed isolated bacteria was 49, as for the non - analyzed ones, it was 90. As the results showed, there was a difference in the bacterial species

producing and nonproducing to hemolysis, and by extracting the value of the value, significant differences were found between the bacterial isolates at the  $p < 0.05$  level.

**CONCLUSION**

Urinary Tract Infections (UTIs) can affect anyone at any age, but some groups are at greater risk, and the main reason women are more likely to get a UTI is due to their female anatomy. The urethra of women is shorter than that of men, and it is also close to the anus, where bacteria can penetrate into the urinary tract. Women's estrogen levels also decline with age, making the urinary tract walls drier and thinner. The protective mucous membrane or mucous layer also becomes less acidic, which reduces its ability to fight infection, which is why treatment with estrogen is recommended to prevent Urinary Tract Infections (UTIs). Urinary incontinence can affect anyone at any age, but it's more common as we get older and when associated with other medical conditions. Therefore, it is common for people with incontinence to have additional problems that contribute to an increased risk of developing a Urinary Tract Infection (UTI).

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