REVIEW ARTICLE

Detection and Characterization of hly,cdtI and stxI genes, in MDR uropathogenic and intestinal E.coli isolates, Hilla city, Iraq.

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Abstract:

Background:. Escherichia coli infections represent a considerable burden in human. Illness caused by E. coli have a significant burden on patients and the healthcare system, so prompt recognition, and appropriate treatment are necessary. Escherichia coli is the first bacterial pathogen in humans responsible for deaths associated with antibiotic resistance

Aim of study: detection of hly, cdtI and stxI genes through molecular analysis in MDR E.coli isolates.

Methods: From November 2021 to May 2022, atotal of 150 urine samples were collected from patients suffering from urinary tract infections were admitted and visit Maternity and Children Babylon Hospital in Al-Hilla city. 100 stool samples were (as control). The identification and antibiotic susceptibility profile of E.coli were done by vitek2 compact system.The isolates were subjected to PCR assays with specific primers for hly , cdtI and stxI .

Results: Only 50(45.5%) E.coli isolates were recovered from the urine samples. In contrast, 60(60%) E.coli isolates from the stool samples. A high rate of MDR was detected among E. coli isolates, where it was(60%) and (53.3%) in the uropathogenic and intestinal isolates, respectively.

The PCR results were showed that MDR uropathogenic E. coli and intestinal E.coli were hardbourd hly gene (46.7%)and (90.6%), respectively. The cdtI gene was detected in (43.4%) of uropathogenic E. coli, and (40.6%) of intestinal E.coli. The stx gene was detected in (16.7%) of uropathogenic E. coli, and (43.7%) of intestinal E.coli.

Conclusion: The presence of a high percentage of toxin in intestinal E.coli is a serious warning because of the possibility of their becoming virulent if the bacteria change their natural habitat or are exposed to a certain stress.

Key words: hly, cdtI, stxI, Escherichia coli

Introduction:

In clinical practice around the world, urinary tract infections (UTIs) are the most prevalent illnesses. Despite various efforts, UTIs continue to impact 150 million people annually around the world, with significant morbidity and huge medical expenditures. A variety of virulence genes must be expressed in a coordinated manner for the UPEC mode of pathogenesis to create long-lasting infections [1][2].

Escherichia coli expressing one or two different forms of Shiga toxin (Stx) are referred to as Shiga toxin-producing Escherichia coli (STEC)[3]. Clinical symptoms of STEC infection include hemorrhagic colitis, which can be fatal, non-immune hemolytic anemia, thrombocytopenia, and acute kidney injury. Other symptoms include mild, watery diarrhea to bloody diarrhea and severe abdominal pain. 5- 15% of STEC infections are said to proceed to HUS[4].

A key component of this bacteria's pathogenicity, hemolysin is released by some strains of E. coli, particularly those that affect the urinary system. Some of these strains can also be found in the colon. It is stored on the chromosome or plasmid that was obtained by horizontal gene transfer. Its pathogenicity is greatly influenced by haemolysin [5].As this protein breaks down red blood cells and creates holes in cell walls, it also helps release vital nutrients like iron, which is necessary for the development and spread of bacterial cells [6].

The class of genetically related bacterial protein toxins known as cytolethal distending toxins (CDT) is capable of halting the growth of multiple cell lines. They are able to activate a signaling pathway in target cells that ordinarily stops the cell cycle from transitioning from the G2 to the M phase, which results in the effect[7].

Materials and Methods

Between November 2021 and May 2022, 150 urine samples from patients with uti who were admitted to the Maternity and Children's Hospital in Al-Hilla city were collected, while 100 stool samples from healthy people served as the control group. All patient and control group samples are drawn from a range of ages and sexes, including both men and women.

Clinical samples were taken from patients using sterile screw-cap containers while handling them safely. According to[8], a loopful (0.01 ml) of urine samples were put on the culture media (MacConkey agar, Blood agar, and EMB agar) and cultured aerobically for 24 hours at 37 °C. After that, biochemical tests were conducted to identify the bacteria.

The 50 isolates of uropathogenic E.coli and 60 intestinal E.coli isolated from healthy individual were subjected to antibiotics susceptibility test by using vitek2 compact system (Biomerieux, France, Card type: GN,AST-N222).. All isolates were examined against 16 antibiotics agents related to 8 antibiotic classes. According to Clinical and Laboratory Standards Institute 2021 recommendations , all results were interpreted and all E.coli isolates were classified as susceptible, intermediate, or resistant to each tested antibiotics agent.

Genomic DNA was extracted from E.coli according to manufacturer instructions of Genomic DNA purification kit (Geneaid, USA). The purity and concentration of DNA for each isolate were measured by Nonodrop instrument (THERMO, USA).

Polymerase chain reaction (PCR) was used to screen for the presence of the toxin genes: hly, cdtI and stxI .In this study all primers were provided from Macrogene company, Korea. Primers details are listed in Table 1.

Table 1: The Primers Sequences used in this study

Genes	Primer sequence (5´- 3´)	Size BP	Reference
Stx I	F:TCTCAGTGGGCGTTCTTATG R:TACCCCCTCAACTGCTAATA	338	Wang *et al*., (2018)
Cdt I	F: CAATAGTCGCCCACAGGA R: ATAATCAAGAACACCACCAC	411	Smith and Bayles *et al.*, (2006)
Hly	F: AGATTCTTGGGCATGTATCCT R: TTGCTTTGCAGACTGTAGTGT	556	Dadi *et al*., (2020)

Results

Only 50 isolates were determined to be E. coli based on culture characteristics and biochemical assays out of 150 collected urine samples, meaning only 110 (73.4%) of those samples were positive for culturing. The fact that E. coli made up 60 (60%) of the 100 control stool isolates and other bacteria made up 40 (40%) implies that it is challenging to culture viruses, fungi, and anaerobic agents. In 110 positive cultures, E. coli was found. In Eosin methylene blue (selective media), 50 (45.5%) of the isolates, including 60 (54.5%) from other bacteria and 35 (70.0%) from females and males, grew probable E. coli. E. coli made up 60 (60%) of the 100 control stool isolates, and other bacteria made up 40%.Table 2.

No. of samples	Positive growth	M.O isolates	No. of isolates (%)
150 urine samples	110(73.4%)	*E. coli*	50(45.5%)
95 Female	88(80%)		35(70.0%)
55 Male	22(20%)		15(30.0%)
		Others	60(54.5%)
		No growth	40(26.6%)
100stool samples	60 (60%) *E. coli*, 40(40%) other M.O		

Antibiotics susceptibility patterns of Escherichia coli

The 50 isolates of uropathogenic E.coli and 60 intestinal E.coli isolated from healthy individual were subjected to antibiotics susceptibility test by using vitek2 system. All isolates were examined against 16 antibiotics agents related to 8 antibiotic classes. All results were interpreted and all E. coli isolates were classified as susceptible, intermediate, or resistant to each tested antibiotics agent. Table(3) and Table (4).

Table (3): Antibiotic susceptibility patterns of 50 of uropathogenic E. coli isolates

No.	Class	Antibiotic	Sensitive		Intermediated		Resistance	
			Isolate No.	%	Isolate No.	%	Isolate No.	%
1.	Cephalosporin	Cefazolin (1st G)	30	60%	2	4%	18	36%
		Cefoxitin (2nd G)	28	56%	4	8%	18	36%
		Ceftazidime (3rd G)	26	52%	2	4%	22	44%
		Ceftriaxone (3 rd G)	23	46%	24	48%	3	6%
		Cefepime (4th G)	30	60%	8	16%	12	24%
2.	Penicillin	Pipracillin-Tazobactam	44	88%	0	0%	6	12%
		Ampicillin	19	38%	5	10%	26	52%
3.	Aminoglycoside	Amikacin	48	96%	0	0%	2	4%
		Gentamicin	37	74%	0	0%	13	26%
4.	Carbapenem	Imipenem	46	92%	0	0%	4	8%
		Ertapenem	44	88%	0	0%	6	12%
5.	Quiolones	Ciprofloxacin	24	48%	0	0%	26	52%
		Levofloxacin	18	36%	0	0%	32	64%
6.	Sulfonamide	Trimethoprim-sulfaamethoxazole	0	0%	0	0%	50	100%
7.	Glycylcycline	Tigecyclin	50	100%	0	0%	0	0%
8.	Nitro furan	Nitrofurantoin	32	64%	0	0%	18	36%
X^2		451.2						
P value		0*						

* Significant difference at P<0.05

This study used the ViteK2 compact system to assess the antibiotic susceptibility of all 50 uropathogenic E. coli isolates, and the results showed that trimethoprim/sulfamethoxazole was completely resistant to all 50 isolates. High rates of resistance to levofloxacin (64%) were also found, as well as to ampicillin (52%) and ciprofloxacin (52%). The isolates› resistance to nitrofurantoin, cefazolin, cefoxitin, gentamycin, piperacillin, imipenem, ceftriaxone, and amikacin ranged from moderate to low (36%, 36%, 36%, 26%, 12%, 12%, 8%, 6%, and 4%, respectively). All isolates, however, are tigecycline-susceptible.

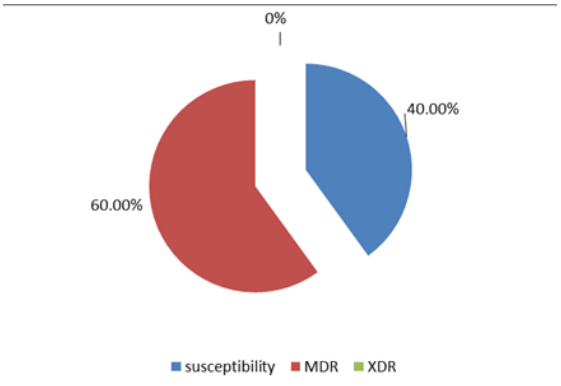
Table (4): Antibiotic susceptibility patterns of 60 of intestinal E. coli from healthy individual.

No.	Class	Antibiotic	Sensitive		Intermediated		Resistance			
			Isolate No.	%	Isolate No.	%	Isolate No.	%		
1.	Cephalosporin	Cefazolin (1st G)	32	53.3%	0	0	28	46.6%	51.92	0**
		Cefoxitin (2nd G)	40	66.7%	0	0	20	33.3%		
		Ceftazidime (3rd G)	44	73.3%	0	0	16	26.6%		
		Ceftriaxone (3rd G)	40	66.7%	4	6.7%	16	26.6%		
		Cefepime (4th G)	60	100%	0	0	0	0		
2.	Penicillin	Pipracillin-Tazobactam	40	66.7%	4	6.7%	16	26.6%	35.07	0**
		Ampicillin	12	20%	0	0	48	80.0%		
3.	Aminoglycoside	Amikacin	44	73.3%	0	0	16	26.6%	0.635	0.426*
		Gentamicin	40	66.7%	0	0	20	33.3%		
4.	Carbapenem	Imipenem	52	86.6%	4	6.7%	4	6.7%	8.57	0.014**
		Ertapenem	60	100%	0	0	0	0		
5.	Quiolones	Ciprofloxacin	52	86.6%	0	0	8	13.3%	0	1*
		Levofloxacin	52	86.6%	0	0	8	13.3%		
6.	Sulfonamide	Trimethoprim-sulfaamethoxazole	52	86.6%	0	0	8	13.3%	---	---
7.	Glycylcycline	Tigecyclin	52	86.6%	0	0	8	13.3%	---	---
8.	Nitro furan	Nitrofurantoin	60	100%	0	0	0	0	---	---
X^2		269.63								
P value		0**								

As can be seen in Table 4, the majority of intestinal E. coli showed significant resistance rates to ampicillin (80%) and cefazolin (46.6%). Cefepime, ertapenem, and nitrofurantoin were all effective against all isolates (100%). High susceptibility rates were recorded for imipenem, levofloxacin, trimethoprim-sulfaamethoxazole, tigecyclin, and ciprofloxacine (86.56%, 86.6, and 86.5 respectively). While isolates showed high susceptibility to ceftazidime, amikacin, cefoxitin, gentamicin, pipracillin-tazobactam, ceftriaxone, and cefazolin, with respective susceptibility rates of 73.3%, 73.3%, 66.7%, 66.7%, 66.7%, and 53.3%.

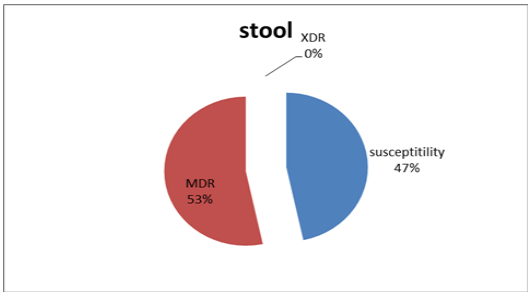
Detection of multidrug resistance of E.coli isolates

According to their resistance to at least one or more of the three or more classes of anti-E. coli antibiotics, MDR E. coli isolates are identified in this investigation [21]. A significant rate of MDR was present in the majority of intestinal and uropathogenic E. coli, with (60%) and (53.3%), respectively. Fortunately, no isolates have ever shown XDR patterns .Figures 1 and 2 are shown.



* Significant difference atP<0.05

Figure (1): The distribution of antibiotics susceptibility patterns of 50 E. coli isolates from urinary tract



*Significant difference at P<0.05

Figure (2): The distribution of antibiotics susceptibility patterns of (60)intestinal E. coli isolates from healthy individual.

Molecular Study of Toxin genes

Detection of hly gene

The presence of (556bp) bands allowed for the detection of the hly gene in 14/30 (46.6%) of the 30 MDR E. coli isolates from urine samples (3a); this gene was also found in 29/32 (90.6%) E. coli isolates from stool samples, as shown in Figure (3b).

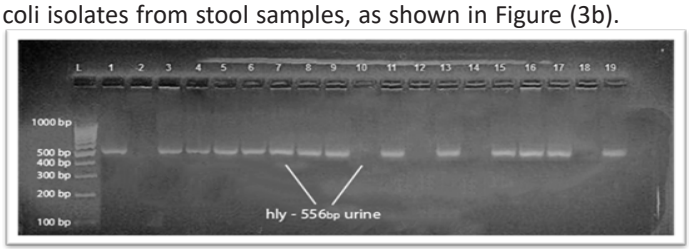


Figure (3a): Gel electrophoresis of PCR product of hly gene at(556bp) of MDR E. coli isolates from urine samples.
L: ladder, lines (1,3,4,5,6,7,8,9,11,13,15,16,17, and 19)positive results for amplification,the electrophoresis was at 70volt for80 min.

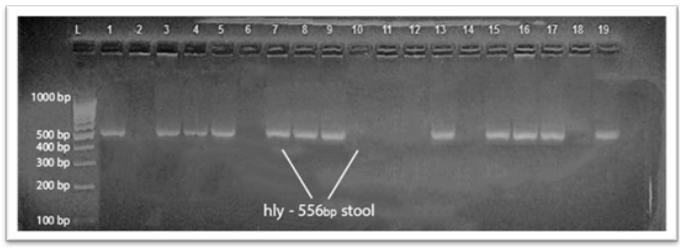


Figure (3b): Gel electrophoresis of PCR product of hly gene at (556bp) of MDR E. coli isolates from stool samples.
L: ladder, lines (1,3,4,5,7,8,9,13,15,16,17and19) positive results for amplification,the electrophoresis was at70 volt for80 min.

Detection of *cdtI* gene

cdtI gene was done for all 30 isolates that previously identified as MDR E. coli isolates from urine samples, PCR is a sensitive and specific method for identification of cdtI gene. The results showed that, 13/30(43.3 %) E. coli isolates were gave positive results for this gene. The positive results were detected by the presence of (411bp) bands as shown Figure (4a), while 13/32 (40.6%) isolates from stool samples as shown Figure (4b).

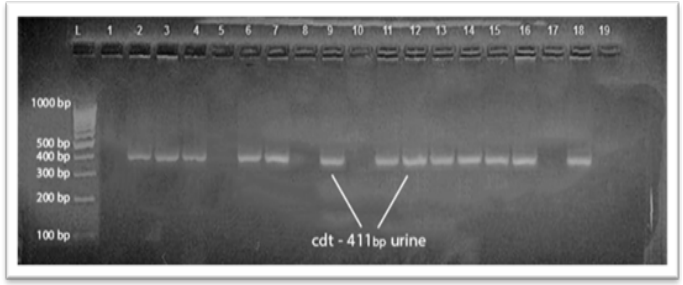


Figure (4a): Gel electrophoresis of PCR product of cdtI gene at (411bp) of MDR E. coli isolates from urine samples.
L: ladder, lines (2,3,4,6,7,9,11,12,13,14,15,16, and 18)positive results for amplification, the electrophoresis was at70 volt for80 min.

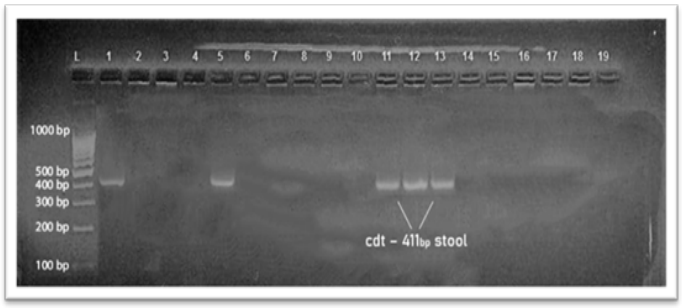


Figure (4b): Gel electrophoresis of PCR product of cdtI gene at (411bp) of MDR E. coli isolates from stool samples.
L: ladder, lines (1,5,11,12 and 13) positive results for amplification, the electrophoresis was at70 volt for80 min.

Detection of *stx1* gene

stx gene was detected in 5/30(16.6 %) MDR E. coli isolates from urine samples, the positive results were detected by the presence of (338bp) as shown in Figure (5a), while this gene was detected in 14/32 (43.%) MDR E. coli isolates from stool samples as shown in Figure (5b).

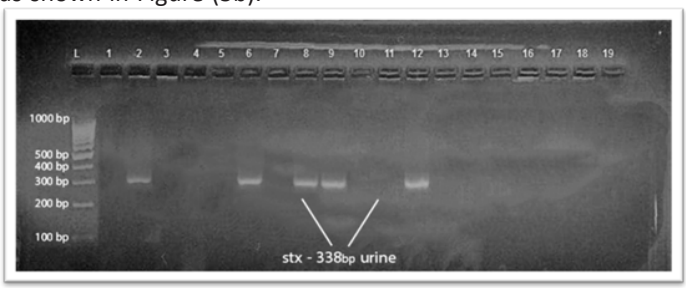


Figure (5a): Gel electrophoresis of PCR product of stx gene at (338bp) of MDR E. coli isolates from urine samples.
L: ladder, lines (2, 6, 8, 9 and 12)positive results for amplification, the electrophoresis was at70 volt for80 min.

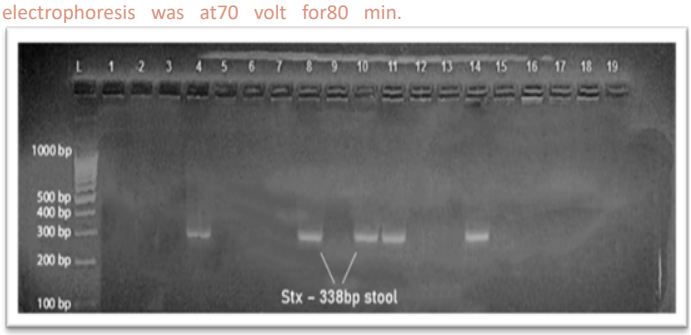
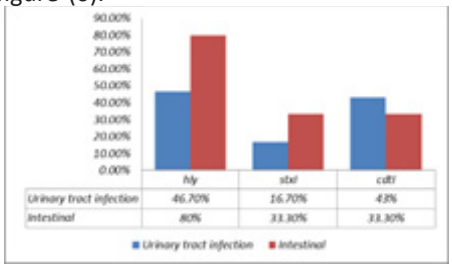


Figure (5b): Gel electrophoresis of PCR product of stxI gene at (338bp) of MDR E. coli isolates from stool samples.
L: ladder, lines (4, 8, 10, 11, and 14)positive results for amplification, the electrophoresis was at70 volt for80 min.

In light of the data discussed above, the distribution of toxins encoded genes among Escherichia coli isolates is shown in the figure (6).



Figure(6) Distribution of Toxin encoded genes among MDR uropathogenic and intestinal E. coli isolates

Discussion

E. coli was identified from UTI infections in (32%) of cases, according to[9], and (76%) of UTI cases, according to [10]. E. coli causes a relatively high percentage (70%) of urinary tract infections, according to research by[11]. The statistics given here were in agreement with [12] findings that E. coli was frequent among UTI infections at a rate of (32%) when compared to past study.

Because of their larger urethras, closer proximity to the anus, and tendency to become pregnant, females are more susceptible to E. coli UTIs than males. Additionally, the germs from the rectum can easily ascend to the urethra and infect it. [13] made this observation. Men in their fifties and older frequently experience urethral obstruction issues, such as benign prostatic hyperplasia, insufficient bladder emptying, and an increase in the prevalence of urinary tract infections (UTIs) as a result [14].

The digestive systems of mammals frequently harbor the bacteria E. coli. The majority of strains pose no risk to the host and may even be helpful when it comes to preventing the growth of pathogenic bacteria in the gut. However, there are E. coli strains that can lead to a variety of illnesses outside of the digestive tract, including as kidney issues, septicemia, neonatal meningitis, and urinary tract infections [15]. According to the World Health Organization (WHO), these bacteria are one of the main pathogens of infections picked up in hospitals. which is the first in the rate of infection with Gram-negative pathogens, and which contributes to a significant portion of infections in hospitals. Infections occurred more frequently as a result of E. coli's numerous drug resistance mechanisms, and bacterial drug resistance gradually increased [14][16].

Antibiotics susceptibility patterns of Escherichia coli

That 90% of E. coli were Trimethoprim-resistant, noted by[17],and their findings are quite similar to those of. Only (87%) of the examined bacteria were resistant to trimethoprim, according to findings by [18].

The isolates from this investigation were less resistant to the majority of tested antibiotics than those from a recent Iraqi study by[19], The majority of E. coli isolates, however, displayed a pattern of strong resistance to amoxicillin, ceftazidime, ceftriaxone, cefepime, and ciprofloxacin, with respective resistance percentages of 95.58%, 92.92%, 92.04%, 90.27%, and 84.96%. While the majority of the E. coli isolates were extremely susceptible to imipenem, the results were in agreement between us.

E. coli resistance to CAZ (58.3%), Ak (25%), CIP (41.7%), LEV (16.7%), IMP (0), and NIT (33.3%) was found in healthy individual stools from Iraqis. [10].

The pattern of susceptibility to antibiotics of E. coli isolates from the urine and excretions in this study was consistent with the findings of [20], who found that E. coli exhibited high resistance to ampicillin and trimethoprim/sulfamethoxazole, while carbapenems and amikacin were the most effective antibiotics against isolates.

Detection of multidrug resistance of E.coli isolates

The findings of [22] are quite comparable to those of this study. Antibiotic resistance, according to [23] research, is the capacity of bacteria to withstand the effects of antibiotics, which can make infections more challenging to cure. Antibiotic resistance known as multidrug resistance (MDR) occurs when bacteria develop resistance to several different antibiotics[24].

Found that 88.09% of the uropathogenic E.coli isolate are MDR and11.90 % of them are XDR isolates, which are higher percentages than recorded in the current study , which is 60% for MDR Uropathogenic E.coli isolates, while no XDR isolates were recorded, this study by [20].

Molecular Study of Toxin genes

Detection of hly gene

It was previously believed that hemolysin, the most broadly distributed toxin produced by E. coli, was an isolated virulence factor [25]. According to [26], infections of the urinary tract, particularly cystitis and acute pyelonephritis, are associated with a higher occurrence of hemolytic E. coli strains.

In addition to the toxin generated, the presence of the hly gene is linked to enhanced pathogenicity of the strains because the strains carrying this gene may also carry a variety of other virulence factors

The hemolysin has also been connected to greater toxicity in human infections. HLY has a lytic effect on macrophages, neutrophils, retinal, and intestinal cells, [27] . According to research by[28], this gene was shown to be prevalent among 14.1% of E. coli strains associated with UTIs. Furthermore, [29] found that the hly gene is only represented in 39% of isolates. According to[30], 33.33% of E. coli isolates contained the hly gene.

Detection of cdtI gene

The cdt operon contains three adjacent genes, cdtA, cdtB and cdtC and

expression of all the genes is necessary for maximum toxin activity while

cdtB acts as an active subunit with DNase I activity, cdtA and cdtC facilitate binding of CDT to identified receptor molecule on susceptible cell and entry of cdtB in to the cytoplasm.

The cdt gene causes irreversible inhibition of cell cycle at the G2/M and produce single nuclear giant cells[31].

In a recent local study in Babylon province, cdt gene was not detected in any isolates that they obtained [10].

Detection of stx1 gene

Shiga toxins are a class of type 2 ribosome-inactivating proteins (RIPs) made by various bacterial species. The toxins have an AB5 structure, which consists of five identical B chains and a catalytic A chain with N-glycosidase activity. This structure allows the toxins to recognize and bind to target cells that have particular carbohydrate moieties.

In humans, the major molecular target which recognizes the Shiga toxins is the Gb3 receptor, which is mainly expressed on the cell surface of endothelial cells of the intestine, kidney, and the brain. This causes these organs to be susceptible to the toxicity of Shiga toxins. When a person is infected by Shiga toxin-producing bacteria, the toxin is produced in the gut, translocated to the circulatory system, and carried to the target cells. Toxicity of the toxin causes inflammatory responses and severe cell damages in the intestine, kidneys, and brain, bringing about the hemolytic uremic syndrome (HUS), which can be fatal [32].

Conclusion: The presence of a high percentage of toxin in intestinal E.coli is a serious warning because of the possibility of their becoming virulent if the bacteria change their natural habitat or are exposed to a certain stress.

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