THE PREVALENCE OF S*tx*1, S*tx*2 GENES AND THEIR SEQUENCING VARIATION IN *E. coli* O157:H7 STRAIN ISOLATED FROM DIFFERENT SOURCES

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Abstract

Background: The two type of Shiga toxin (Stx1, Stx2) is the most important virulence factors associated with the pathogenicity of E. coli O157:H7. Aim: The current study aimed to detect these toxins and study their sequence alignment in E. coli O157:H7 isolated from different sources. Materials and Methods: Stx1 and Stx2 genes were detected by conventional PCR technique using specific primers. Sequencing of genes was performed by using Sanger sequencing methods. Results: The results indicate that the presence of the Stx1 gene is low in all the isolates compared to the gene Stx2 which was the most present in the studied isolates. Diarrheal isolates were the most isolates that carry Stx1 and Stx2 genes, and the least are the environmental isolates. Gene sequential analysis results showed that both genes, Stx1, and Stx2, belong to the E.coliO157:H7 strain with a match rate of 99% compared to the NCBI database, ten new strains of E.coliO157:H7 were registered in the NCBI database and each of them was given its serial number. The genetic changes that were detected in new strains varied between transversion and transition, which led to the occurrence of different types of point mutations, including missense and silent mutations. Conclusion: The presence and spread of Stx1 and Stx2 genes vary depending on the source of E.coliO157:H7 strain isolation and the genetic sequence of these genes can change as a result of different mutations.

Keywords: *E. coli O157:H7, Shiga toxin, Stx1 gene, Stx2 gene, Mutations*.

INTRODUCTION

Shiga-like toxins production is a key virulence factor associated with the pathogenicity of E. coli O157:H7 which produces one or two types of Shiga-like toxins, stx1 and stx2 responsible for most clinical symptoms of its diseases (1,2). This toxin is called Shiga because it is structurally and functionally similar to the toxin of Shigella dysentery, except that it differs in one amino acid and is also called Verotoxin, Cytotoxin (3). The term Shiga -like toxins was used until it was proven and confirmed that they are the same as Shiga toxins (4). Shiga toxin is a structural protein consisting of two units, each of which has a different molecular weight, they are bound to glycolipids present on the surface of host cells especially Globotriaosylceramide (Gb3) (5). The two types of Shiga toxin (Stx1, Stx2), are structurally similar but different antigenically (6).

Shiga toxin is very toxic to cells (potent cytotoxic) and has a major role in causing gastroenteritis, bloody diarrhoea in humans

that may be complicated by hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) which is the main cause of acute renal failure in children. It damages the intestinal epithelial cells and kidneys (7).

They found that Stx2 is about 400 times more virulent compared to Stx1 in an experiment conducted on mice, as Stx2 has a fast and strong effect on the Gb3 receptors that are abundant in the epithelial tissues of the kidneys and cells of the nervous system, as it works to destroy these cells and cause pathological events (8). The pathological signs that appear on the host as a result of the effect of this to are high fever and bloody diarrhoea as a result of Hemolytic Colitis (HC), Hemolytic Uremic Syndrome(HUS), depending on the amount of toxin and its site of action (9). Variation in disease severity among E. coli O157:H7 strains may result from differential expression of stx2, which appears to be more responsible for serious complications in HUS than those only stx1 producing. (10,11). The current study aimed to detection of Shiga toxins types (stx1 and stx2) and study the variation in their gene sequence in E. coli O157:H7 strain isolated from different pathological, food and environmental sources.

Materials and Methods

E.coli O157:H7 isolates: Fifty isolates of E.coli O157:H7 strain were used in the current study isolated from different sources (Diarrhea, urine, food, river water, and sewage water, ten isolates from each source) previously diagnosed in the Department of Biology, College of Science, University of Mosul.

Primers: The primers used in the current study were supplied by (Macrogen \Korea) (Table 1).

Table 1. Forward and reverse primer blast designed for tested genes.

Genes	Primers Sequence	size (bp)
SLTI-F	ACA CTG GAT GAT CTC AGT GG	614
SLTI-R	CTG AAT CCC CCT CCA TTA TG	
SLT2-F	CCA TGA CACA CGG ACA GCA GTT	779
SLT2-R	CCT GTC AAC TGA GCA CTT TG	

DNA Extraction: Genomic DNA of the isolates was extracted using the specialized kit (Presto™ extraction kit. Mini DNA Bacteria Kit Geneaid. USA). The purity and concentration of the extracted DNA were measured using a BioDrop spectrophotometer (Cambridge\ England), the primer supplied by the company (Macrogen \Korea).

Detection of Stx1, and Stx2 genes: Stx1 and Stx2 genes were detected by conventional PCR technique using specific primers(12). The reaction mixture described in table 2 and The amplification protocol was carried out as described previously published study conducted by Ranjbar et al. (2018)(13) as described in table 3.

Table 2. Mixture of the interaction.

Components	Concentration
Taq pre master mix	12.5µl
Forward primer	(1µl)
Reverse primer	(1µl)
DNA	2.5 µl
Free nuclease water	8 µl
Final volume	25µl

Table 3. tested genes Amplification Program

Steps	*STX*1			*STX*2		
	˚C	m:s	Cycle	˚C	m:s	Cycle
Initial Denaturation	94	10:00	1	95	5:00	1
Denaturation	94	00:45	35	95	00:45	35
Annealing	58.9	00:45	35	58	00:45	35
Extension	72	11:00	35	72	00:45	35
Final extension	72	5:00	1	72	7:00	1

Agarose Gel Electrophoresis: After the PCR process, electrophoresis was adopted to confirm the presence of amplification (Agarose gel 2%, Electrical power 80 V, for 45min) PCR products were loaded directly. The safe red-stained bands in gel were visualized using a Gel imaging system.

Gene Sequencing: Sequencing of genes was performed by (Macrogen \Korea), using Sanger sequencing methods. Five isolates carrying both genes (Stx1 and Stx2) belonging to five isolate sources (Diarrhea, urine, food, river water and sewage water) were selected for this test. Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center for Biotechnology Information (NCBI).

Results

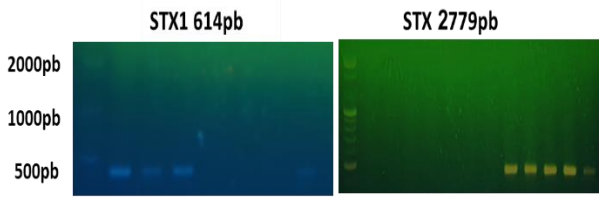
The number and percentage of isolates that carry the Stx1and Stx2 genes in the studied isolates from their various sources (diarrhoea, urine, food, river water, sewage water) shown in(Table 4). It is noted that the gene Stx1 was present in 20% of the total samples, while the gene Stx2 was present in 40% of these samples, and the presence of both genes varied according to the source of the isolation.

Table 4. Stx1 and Stx2 gene distribution in the studied isolates from different sources.

Isolates Source (No.)		Type of gene			
		Stx1		*Stx2*	
		+	-	+	-
Clinical	Diarrhea (10)	(%40)4	(%60)6	(%50)5	(%50)5
	Urine (10)	(%10)1	(%90)9	(%40)4	(%60)6
Environmental	River water (10)	(%10)1	(%90)9	(%30)3	(%70)7
	Sewage water (10)	(%10)1	(%90)9	(%30)3	(%70)7
Food (10)		(%30)3	(%70)7	(%50)5	(%50)5
Total		(%20)10	(%80)40	(%40)20	(%60)30

The Stx1 and Stx2 gene investigating bands using PCR technique, as it is noted that the bands of these genes appeared in some isolates and did not appear in other isolates (Figure 1).

Figure 1. tested genes bands using PCR gel electrophoresis technique.



The sequential analysis of the Stx1 and Stx2 genes which carried out by the Sanger sequencing method for the five selected isolates, and it was found that both genes, Stx1, and Stx2, belong to E.coliO157:H7 strain with a match rate of 99% compared to the NCBI database (Table 5 and Table 6).

Table 5. Gene sequencing results of the Stx1 gene for the selected E.coliO157:H7 isolates compared to the database NBCI

No.	Type of substitution	Location	Nucleotide	Amino acid change	Predicted effect	Sequence ID with compare	Source	Sequence ID with Submissions	Identities
1	Transvertion	271	C\ G	Leucine\ Valine	Missense	ID: EF079675.1	Sewage water	OP785749.1	99%
	Transition	285	A\ G	Arginine\ Arginine	Silent				
2	Transition	85	T\C	Phenylalanine \ Proline	Missense	ID: EF079675.1	Urine	OP785751.1	99%
	Transition	86	T\C	Phenylalanine \ Proline	Missense				
	Transvertion	358	G\T	Valine\ Phenylalanine	Missense				
3	Transvertion	271	C\ G	Leucine\ Valine	Missense	ID: EF079675.1	Food	OP785750.1	99%
	Transition	285	A\ G	Arginine\ Arginine	Silent				
	Transition	458	T\C	Serine\ Serine	Silent				
4	Transvertion	271	C\ G	Leucine\ Valine	Missense	ID: EF079675.1	Stool	OP785752.1	99%
	Transition	285	A\ G	Arginine\ Arginine	Silent				
	Transvertion	370	G\ C	Glycine\ Arginine	Missense				
5	Transition	85	T\C	Phenylalanine \ Proline	Missense	ID: EF079675.1	River water	OP785753.1	99%
	Transition	86	T\C	Phenylalanine \ Proline	Missense				
	Transvertion	516	G\T	Leucine\ Leucine	Silent				

Table 6. Gene sequencing results of the Stx2 gene for the selectedE.coliO157:H7 isolates compared to the database NBCI

No.	Type of substitution	Location	Nucleotide	Amino acid change	Predicted effect	Sequence ID with compare	Source	Sequence ID with Submissions	Identities
1	Transvertion	277	A\T	Isoleucine\ Phenylalanine	Missense	ID: JQ411011.1	Sewage water	OP785754.1	99%
2	Transvertion	311	T\G	Valine\ Glycine	Missense	ID: JQ411011.1	Stool	OP785757.1	99%
	Transvertion	312	T\G	Valine\ Glycine	Missense				
3	Transvertion	402	C\G	Serine\ Arginine	Missense	ID: JQ411011.1	River water	OP785758.1	99%
	Transition	577	T\C	Phenylalanine \ Leucine	Missense				
4	Transvertion	311	T\G	Valine\ Glycine	Missense	ID: JQ411011.1	Urine	OP785756.1	99%
	Transvertion	312	T\G	Valine\ Glycine	Missense				
	Transvertion	419	A\C	Glutamine\ Proline	Missense				
5	Transvertion	277	A\T	Isoleucine\ Phenylalanine	Missense	ID: JQ411011.1	Food	OP785755.1	99%
	Transition	363	A\G	Serine\ Serine	Silent				

Discussion

In the current study, both the Stx1 and Stx2 genes of E.coli H7:O157 which are responsible for the virulence of these bacteria were detected using the PCR technique, The results show that the presence of the Stx1 gene is low in all the isolates under study by (10) isolates (20%), while the gene Stx2 is the most present in the studied isolates with several (20) isolates (40%) and this agrees with the study conducted by Jebur (2020) (14), where the percentage of the Stx2 gene was (30%) and Stx1 gene (15%). It also agrees with the study Abdulrazzaq et al., 2021 (15) conducted in Mosul, where the percentage of the Stx1gene was (33.3%), while the Stx2 gene was (44.4%).

The results also showed that diarrheal isolates were the most isolates that carry Stx1 and Stx2 genes, and the least are the environmental isolates, where only one isolate carried the gene Stx1 (10%) and (3) isolates carrying the gene Stx2 (30%) for both river water and sewage water isolates.

Stx2 has greater diversity compared to Stx1, which led to the emergence of many types of

toxins (16). Stx1 remains cell-bound and stored in the periplasmic region, while Stx2 is released from bacterial cells, so it is usually detected at a higher standard(17,18).

Sequential analysis of the Stx1 and Stx2 genes was carried out by the Sanger sequencing method for the five selected isolates, and it was found that both genes, Stx1, and Stx2, belong to E.coliO157:H7 strain with a match rate of 99% compared to the NCBI database (Table 5, 6).

Based on the results of the sequential analysis of both genes, ten new strains of E.coliO157:H7 were registered in the NCBI database and each of them was given its serial number, as it was found that several different mutations occurred in the nitrogenous bases of the Stx1 and Stx2 genes of the five studied isolates compared to the standard strains of these bacteria in the NCBI database.

The genetic changes that were detected varied between transversion and transition, which led to the occurrence of different types of point mutations, including missense mutations that lead to a change in the amino acid encoded, and silent mutations in which the amino acid does not change. The mutations that were investigated occurred in a different locations within the same gene.

The numbers and types of genetic changes and mutations that occurred on the Stx1 and Stx2 genes differed for each type of studied isolates, and the Stx1 gene was the most affected, as the five isolates had 14 mutations compared to the Stx2 gene, which the isolates had 10 mutations, and the sewage isolate were the least exposed to mutations compared to the rest.

The mutation caused a change in the nucleotide sequence in a short region of a genome, and this results in phenotypic change which may vary on the severity and location of the mutation. The mutations may result from errors during DNA replication or from exposure to mutagens (like chemicals and radiation). Spontaneous mutations occur at a rate of 1 in 105 to 108 and contribute to random population variation. Mutations can cause changes to protein expression and function, and also can produce changes in structural or colony characteristics or loss in sensitivity to antibiotics (19,20).

Conclusion

The presence and spread of Stx1, and Stx2 genes vary depending on the source of E.coli O157:H7 strain isolation and the genetic sequence of these genes can change as a result of different mutations. The genetic changes that were detected varied between transition and transition.

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