



## **Role of Toxins of Uropathogenic *Escherichia coli* in Development of Urinary Tract Infection**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Three clinical syndromes including diarrhea/enteritis, urinary tract infection and septicemia/meningitis can be found as a result of infection with each of the *E. coli* pathotypes. Uropathogenic *Escherichia coli* toxins are one of the most important factors in the spread of these infections, including urinary tract infections, which we will review in this study. The first CDT toxin (Cyclomodulins) was reported in *E. coli* strains in 1987; CDT toxins show a new function and act on DNA. This unique CDTs has unlocked novel questions in the field of Toxicology. The *E. coli* strains involved in UTIs often produce exotoxins such as hemolysin, cytotoxic factor type 1 (CNF1), and colonization factors. Enterotoxigenic *E. coli* (EAEC) toxins include heat-resistant enterotoxigenic toxin (EAST1) plasmid, a potent agent for diarrhea, encoded by a plasmid (Pet), which has cytotoxic activity against cultured intestinal cells and red blood cells,

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toxin Pic and ShET1 Toxins that have intestinal toxicity. EHEC toxins include Shiga Toxin (Stx) and Verotoxin (VT) and Enterohemolysin. Also, EAST1 was reported for the first time in EAEC strains, and this toxin is also present in EHEC strains. ETEC enterotoxins belong to one of the two heat-sensitive enterotoxins (LTs) and heat-resistant enterotoxins (STs). EPEC toxins include EspC enterotoxin. VAT toxin is a known cytotoxin that expresses through UPEC over a systemic infection. CysK also contributes to the expression of UPEC toxins and the cause of toxicity and cellular inhibition caused by UPEC infections.

**Keywords:** *Escherichia coli*; toxins; UPEC; UTI; Urinary tract infection.

## 1. INTRODUCTION

### 1.1 *Escherichia coli*

*Escherichia coli* belongs to genus *Escherichia*. The name of the genus *Escherichia* is due to Theodor Escherich, who carried out basic studies on neonatal feces and discovered the organism in 1885. *Escherichia coli* is the most common facultative anaerobic species of Enterobacteriaceae family found in the human intestine. *Escherichia coli* are most widely studied species of Enterobacteriaceae when compared to any other bacteria from the same family [1].

### 1.2 Diseases

*E. coli* is generally considered pathogenic and is capable of conferring the following three clinical syndromes, which include diarrhea/enteritis [2,3], urinary tract infection and septicemia/ meningitis [4,5].

### 1.3 Diarrhea

*E. coli* pathotypes that cause diarrhea are globally distributed and classified according to their pathogenic properties, and each group develops a different mechanism of disease. The adhesion properties of the small and large intestinal epithelial cells are encoded by the genes that are on the plasmid. Genes that encode toxins are also often found on the plasmid or phage [6].

### 1.4 Urinary Tract Infection

Urinary tract infection is one of the most commonly reported extra-intestinal infections by *E. coli* and is caused by uropathogenic *E. coli* (UPEC). As a matter of fact, *E. coli* is considered as the main perpetrator of UTI. A subset of *E. coli* causing pyelonephritis and cystitis are distinct from commensal *E. coli*, which are located in the lower part of the colon [7].

### 1.5 Septicemia/Meningitis

*E. coli* are capable of causing extra-intestinal infections, like meningitis and septicemia called meningitis-associated *E. coli* (MNEC).

*E. coli* pathotypes are the prime causative agents of meningitis in neonates resulting in 15-40% of mortality rates. These Pathotypes also cause neural defects in many infants [1].

## 2. CYTOLETHAL DISTENDING TOXINS (CDTs)

The first Cytolethal distending toxin was reported in 1987 in strains of pathogenic *E. coli*. The CDTs family consists of bacterial protein toxins produced by various gram-negative bacteria like *Escherichia coli* [8], *Aggregatibacter actinomycetemcomitans* [9], *Haemophilus ducreyi* [10], *Shigella dysenteriae* [11], *Campylobacter* spp. [12], *Helicobacter* spp. [13], and *Salmonella enterica* [14]. CDT and colibactin (a well-known genotoxin, a hybrid of a peptide-polyketide produced by commensal *E. coli* strains) are among the first bacterial genotoxins that have unique properties damaging the target cell DNA [15].

### 2.1 Structure and Activity of CDT

CDT is a product of an operon that encodes three proteins. All these proteins are required to produce a complete toxin. These three proteins are encoded by *cdtA*, *cdtB*, and *cdtC* genes that produce CdtA, CdtB, and CdtC proteins. The subdivisions of CdtA, CdtB, and CdtC have molecular weights of 25, 30 and 20 kDa respectively [15,16]. The protein sequence of the CDT gene product is not similar to any known protein, but BLAST has revealed, DNase I-like enzymatic activity in the subunit of CdtB toxin CDTs of *Escherichia coli* and *Campylobacter jejuni*. Studies have illustrated that the CDT toxin of *Haemophilus ducreyi* induces a fracture in two DNA strands after entering the mammalian cells.

Recent, studies on holoxin crystalline structure of CDT *Haemophilus ducreyi*, disclosed the presence of an enzyme belonging to the DNase I family (CdtB), which is linked to two domains of ricin (CdtA and CdtC) [16]. The bacterial toxins attack the cell membrane or different targets within the cytoplasm, but CDTs show a new function and act on DNA [16]. This unique CDTs performance has created new questions in the field of Toxicology. For example, how does the CDT active subunit reach its target in the cell nucleus? What is the mechanism of molecular transfer of CdtB to the cell nucleus?

## 2.2 The Entry of CDT into the Cell

CDT is the first known bacterial toxin that targets the cell's core. This toxin must first be attached to the cytoplasmic membrane to enter the cell. In the study of the route, CDT injection has been used more from *E.coli* (EcCDT-II), *Haemophilus ducreyi* (HdCDT), and *Aggregatibacter actinomycetemcomitans* (AaCDT) (Image) [15,17].

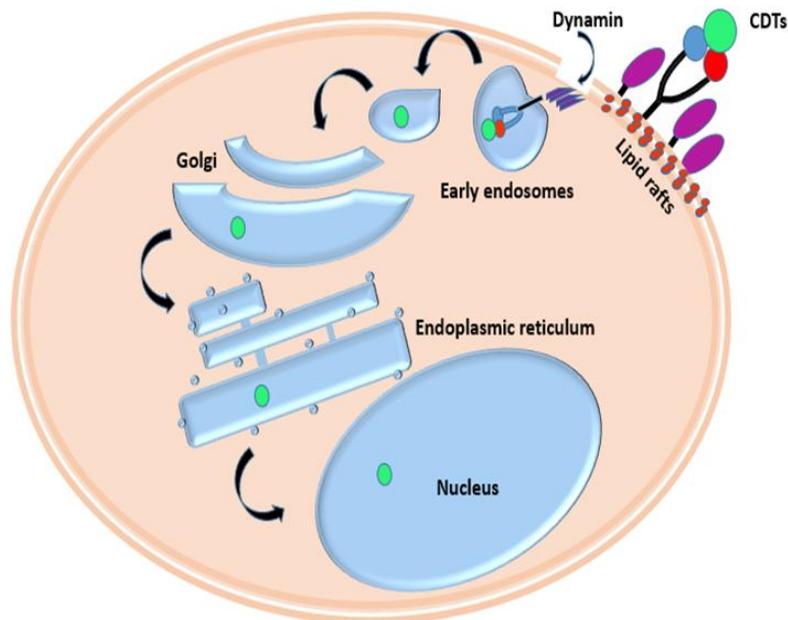
## 2.3 CDT Toxin in *Escherichia coli* Pathovars

There are several *E. coli* toxins called cyclomodulins which interferes with the

eukaryotic cell division cycle [18]. Four types of cyclomodulins have been identified in *E. coli* [19]:

- 1- Cytotoxic Necrosis Factors (CNFs)
- 2- Cycle-inhibiting factor
- 3- CDT, which has 5 types: I to V.
- 4- Colibactin

Five types of CDT toxins have been reported in *E. coli*. Like other CDTs, the *E. coli* toxin is composed of three subunits encoded by the *cdtA*, *cdtB*, and *cdtC* genes. All three genes are located on an operon. CDT-III is located on a large Virulence plasmid (pVir). The genes encoding CDT type I, II, IV, and V are on the chromosome. Čurová et al. in 2014, examined the ExPEC (Extraintestinal pathogenic *E. coli*) strains obtained from blood infections due to the presence of CDT genes. They showed that *cdt-I* and *cdt-IV* genes were detected in one strain of 80 strains [19]. In a study by Hinenoya et al in Japan on 362 fecal specimens obtained from patients with diarrhea, they found the *cdtB* gene in 35 samples (9.7%) by using PCR. Of the 35 samples, 21 samples were positive from the *cdt-I* gene, 3 for the *cdt-II* gene, 4 for *cdt-III* and 3 for the *cdt-V* gene. Interestingly, none of these strains of *E. coli* were positive for the virulence



**Fig. 1. CDT entry path. The binding of CDT to the cytoplasmic membrane of the target cell is dependent on the presence of intact lipid rafts and the toxin is introduced into the initial and delayed endosomes by endocytosis-dependent dynamin. Subsequently, the CdtB subunit is transferred to the Golgi apparatus and reversely transmitted to the endoplasmic network (ER) and from the endoplasmic network to the nucleus**

genes found in the conventional pathovars of diarrhea (DEC) and were not present in these pathovars [20]. Bouzari et al. [21] in Iran in 2005, revealed the role of *E. coli* as diarrhea causative agents (DEC) and have identified the *cdt-B* gene in 37.5% isolates. Kim et al. [22] in South Korea, examined 366 isolates of EPEC in patients with diarrhea with the *cdt-B* gene. Their results showed that 2.7% of isolates have this gene. The identified toxin genes were *cdt-I*, *cdt-IV* and *cdt-V*.

## 2.4 Cytotoxic Necrotizing Factor (CNF)

*E. coli* generate all types of extra-intestinal infections, such as neonatal meningitis, septicemia, and urinary tract infections (UTIs). *E. coli* strains involved in UTIs often produce exotoxins such as hemolysin, cytotoxic necrotizing factor type 1 (CNF1), and colonization factors. The genes encoding exotoxins and colonization factors are located in the region of the chromosomal DNA called pathogenicity islands [23]. The small GTP binding proteins in the Rho family are one of the common goals of bacterial toxins. GTPases are involved in the regulation of several cellular processes, such as cellular rearrangement, endo and exocytosis, cell proliferation and apoptosis [24].

## 2.5 Cnf1 Gene

The Uropathogenic *E. coli* (UPEC) CNF1 protein is encoded by a 3042 bp gene, which comprises of 1014 amino acids (approximately) [25]. Some ETEC strains found in animals produce CNF2, which is similar to CNF1 (86% amino acid analogy). The *cnf1* gene found in PAI *E. coli* J96 strains has been widely studied [26]. PAI V containing the *cnf1* gene, located between the alpha-hemolysin (Hly) operon and the adhesins encoding genes related Pap (prs). Almost all of the Uropathogenic *E. coli* strains to produce CNF1 (about 30% of the *E. coli* strains are involved in urinary tract infections) contain the alpha-hemolysin gene (*hly*) in up-stream of the *cnf1* gene. It is possible that CNF1, along with Hly hemolysin, is involved in a virulence mechanism that makes the benefits for the bacteria. The CNF1 does not contain any signal sequence and therefore is not secreted through the type 2 secretion system. Since anti-CNf1 antibodies are found in patients infected with *E. coli*, it is likely that CNF1 is secreted in vivo (in the human body), but until now its mechanism of secretion remains unknown [27].

## 2.6 CNF1 Effects on Eukaryotic Cells

CNF1 causes tissue damage and degenerate the epithelial cells [28]. The first CNF1 was introduced into rabbit skin as a dermonecrotic toxin through injection. CNF1 also has a lethal effect into mice [29]. Further studies on CNF1 revealed its role in the stimulation and formation of thick actin stress fibers and actin relation membrane folding confirming its role in influencing the actin skeleton by regulating Rho GTPase [30]. The effects of CNF1 on the actin skeleton of Hep-2 cells are remarkable. Fibroblast cells such as Vero show the formation of thick actin stress fibers, while epithelial cells such as HEp-2 exhibit more filopodia and lamellipodia. The CNF1 toxin in HEp-2 cells also produces large vacuoles [24]. The *cnf1* gene has been identified in the *E. coli* K1 strains involved in meningitis [31]. The CNF1 entry is shown in the image. CNF1 enters the host cell through receptor-dependent endocytosis (Fig. 1) [32].

## 2.7 Molecular Mechanism of the CNF1

CNF1 toxin induces cellular actin skeleton through permanent stimulation of Rho GTPases. CNF1 toxin increases the deamination of glutamine 63 to glutamic acid in Rho or glutamine 61 to glutamic acid in Rac and Cdc42. Glutamine 63 in Rho (or Glutamine 61 in Rac and Cdc42) is indispensable for the intrinsic activity of GTPases induced by the Rho GTP binding proteins, due to the activating protein of GTPases. As a result, the Rho GTPases deformed by toxin are locked in the active mode where the GTP is connected (Fig. 2) [32].

## 2.8 CNF1 Toxin Structure

The N-terminal of CNF1 contains a cell-binding portion region. The central area of the CNF1 toxin, which contains amino acids 190 to 720, contains the hydrophobic structure and can play the role of the domain membrane transducer (domain T). The catalytic domain CNF1 is located at the carboxyl end (amino acid 720 to 1014) (Fig. 3) [32,33].

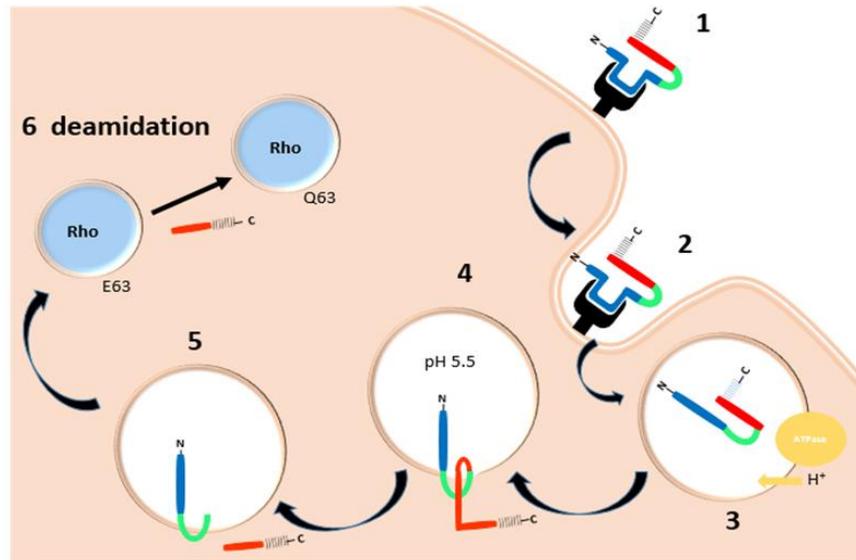
## 2.9 Enteroaggregative *E. coli* (EAEC) Toxins

### 2.9.1 Enteroaggregative heat-stable toxin1

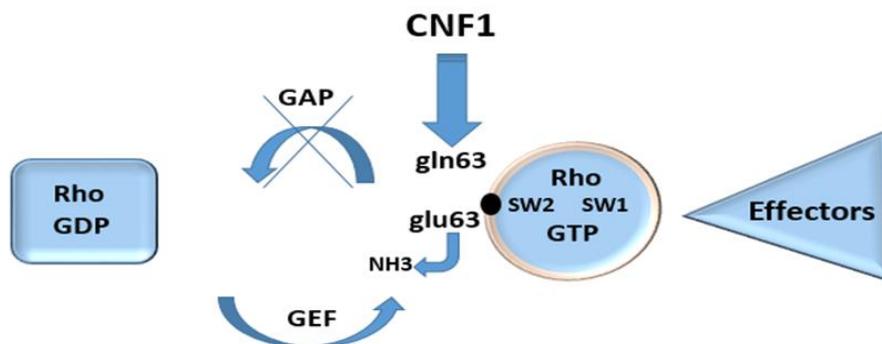
Vial et al. has demonstrated that EAEC is pathogenic to rabbits and carries plasmid-encoded virulence genes. The first virulence

factor that contributes to the development of diarrhea is EAST1. EAST1 is a peptide consisting of 38 amino acids and is homologous to ETEC heat-stable enterotoxin and guanylin signal peptide. This toxin was originally obtained from a filtered culture of strain 2-17.

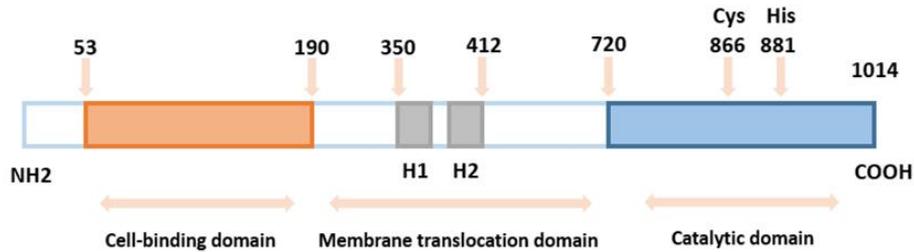
Subsequently, *astA* gene (EAST1 encoder) was present in 41% of EAEC strains. However, this gene also exists in 100% of the strains of O157:H7 (EHEC), 41-6% of the other causative diarrhea and 38% of the normal flora of the *E. coli* [34].



**Fig. 2. CNF1 entry into the cell. (1) The CNF1 toxin is connected to the 67-kDa laminin receptor via its N-terminal. (2) receptor-dependent endocytosis. (3), acidification of the initial endosome. (4), CNF1 deformation due to the acidic environment and entry of the transitory domain to the membrane of the vesicles. (5), the breakdown of toxin by the serine protease, and release of the C-terminal of the portion, which is catalytically active. (6), CNF1 deamidated Rho proteins in Gln63 / 61**



**Fig. 3. The molecular mechanism of CNF1 on Rho GTP-binding proteins. This image shows the mechanism of the CNF1 / CNF2 operation of *E. coli* on the activation and inactivation of GTPases cycle. GEF, Guanine Exchange Factor, GAP, GTPases Activating Protein, SW1, Second Switch 1, SW2, Second Switch 2. CNF1 / CNF2 modify through the deamination of Rho glutamine 63 (61 in Rac, Cdc42) and thus inhibits the activity of GAP and GTPases, and therefore the connected GTP active state remains active permanently and effects The next one will appear (Figs. 2 & 3)**



**Fig. 4.** The schematic image of *E. coli* CNF1. The numbers displayed above the arrows indicate the position of the amino acids

### **2.9.2 Plasmid-encoded toxin (Pet)**

Pet is a serine protease that is a type of autotransporter proteins. In addition to the role of enterotoxins, Pet has cytotoxic activity against cultured intestinal and red blood cells. Cytotoxic activity is probably due to the intracellular mechanism induced by the destruction of the membrane protein of spectrin. *In vitro* studies have shown that purified toxin induces prolongation of cells and resulting in exfoliating the cells. *Pet* gene is among a group of specific locus of pathogenicity on the PAA plasmid in O42 strains [35].

### **2.9.3 Protein involved in intestinal colonization (Pic)**

Pic toxin is a 116-kDa protein whose gene encodes on the chromosome of EAEC [36], UPEC [37] and also in *Shigella flexneri* [38]. This protein is an extracellular serine protease capable of mucolytic, serum resistance, and hemagglutination activity. Protease is synthesized as a large precursor and is processed by secretion mechanism during secretion [39]. It has also been shown that Pic protects the protease activity on complement components and improves EAEC function in the intestine [40].

### **2.9.4 Shigella enterotoxin1 (ShET1)**

The Pic protein gene is located on the strand of the toxin genes ShET1 (set1A and set1B) and contain the genetic information for the same which is toxic to the intestine [41]. In rabbit model, it has been shown that this toxin causes the accumulation of fluids in the ileum of the intestine [42]. ShET1 was first described in *Shigella flexneri* 2a [43]. The position of this gene in both bacteria is on the field against the

mucinase gene (Pic). The Active ShET1 has a subunit A and several subunits B (A<sub>1</sub>-B<sub>n</sub>) [44].

### **2.9.5 EAST1**

EAST1 was first reported in EAEC strains [45], but several studies have confirmed its presence in EHEC strains as well [46]. A demonstrative study on many O157: H7 strains, disclosed the presence of *astA* gene in all the strains (the EAST1 toxin-encoding gene) [47].

### **2.9.6 ETEC toxins**

ETEC enterotoxins belong to one of the two heat-labile enterotoxins (LTs) and heat-stable enterotoxins (STs) [48,49]. ETEC strains may only express LT or ST alone or both. LTs are a category of enterotoxins that have a close structural and functional relationship with Cholera Enterotoxin (CT) and are found predominantly in human isolates. LTs enterotoxins are divided into LTI (similar to cholera toxin) and LT II groups. STs are small singleton toxins, which include two STb and STa classes (which differ in structure and function) [50]. STb is associated with animal disease and is composed of a peptide. STb increases the concentration of intracellular Ca<sup>2+</sup>, stimulates the release of prostaglandin E<sub>2</sub>, and stimulates serotonin secretion, which releases the ions [51].

### **2.9.7 EPEC toxins**

The Enteropathogenic *E. coli* secreted protein (EspC) Enterotoxin is an autotransporter protein in EPEC strains [52]. The EspC molecule has an amino acid analogy with the members of the self-transmissible IgA family proteases (such as the IgA protease of the *Neisseria gonorrhoeae*, the IgA protease of *Haemophilus influenzae* and Pic in the EAEC [53].

### **2.9.8 VAT toxin**

The vacuolating autotransporter toxin, expressed during the systemic infection with UPEC, very common and is a highly protected immunogenic protein that belongs to the protease family of serine protease from Enterobacteriaceae and is secreted by UPEC during infection [54]. The regulation of the expression of the gene in the VAT represents a process in which it is directly suppressed by the H-NS (histone-like nucleoid structuring proteins) transcription regulator and, on the other hand, is up-regulated and expressed through the downstream of the *vatX* gene [55].

### **2.9.9 The role of CysK in expressing UPEC toxins**

Recent studies have illustrated the effectiveness of interaction between CysK / CDIA-CT and the formation of toxicity and cellular inhibition caused by UPEC infections. The oligomeric states of proteins and protein-protein complexes involved with the Contact-Dependent Growth Inhibition (CDI) system are obtained through thermodynamic and kinetic parameters for the first time in the current study [56]. Recent studies have shown the effectiveness of interaction between CysK / CDIA-CT and the formation of toxicity and cellular inhibition caused by UPEC infections. According of these information, UPEC536 infection due to CDI activity is because of the role of CysK in two functional pathways, including modulating the immune complexes of inhibitor cells and activating toxins in target cells [57].

### **2.9.10 Adjustment of gene expression through epigenetics in UPEC toxins**

In *E. coli* and other prokaryotes, as well as in eukaryotes, DNA methylation is an significant epigenetic process that takes place after the replication by a class of DNA methyltransferase (MTases) enzymes, according to which many of the relevant gene expression is organized within the cell [58,59]. Among them, DNA adenine methylase (Dam) plays a vital role in DNA repair [60], regulation of gene expression [61,62] and stimulation of the SOS response as part of the cell cycle [63]. Also, DAM affects the expression of genes that contribute to the pathogenicity of bacteria that is present in different strains. On the other hand, the mutation in the EHEC Gene DAM increases the UPEC adhesion to mammalian culture cells *in vitro*, which is contrast to the wild EHEC, indicating an increase in the expression

of its mutant type. However, in the absence of proper DAM, we will show the alternate pathogen pathways due to increased gene expression in toxins and connectors [64].

### **2.9.11 Transfer of UPEC toxin into host cell**

In gram-negative bacteria, there are processes for manipulating the defense mechanism of the host cell, which causes the infection and prolongs the infectivity. Bacterial signals that alter the function of the host cell are transmitted to the host cell by membrane vesicles (MVs). During infections, MVs are responsible for the transmission of toxins and infectious agents to the host cell and also protect the bacteria against the host immune system, which further causes bacterial colonization. These vesicles allow the accumulation of insoluble components, offers protection against external enzymes and antibodies, as well as create a mechanism for direct binding to the host cell. In this context, studies on UPEC of strain 536 shows that MVs contain hemolysin toxin proteins, and in other UPEC strains, they carry another toxin, including CNF1, whose purpose is to provide Rho GTPase for host cell signaling for acute induction of response Inflammation. It is also known that MVs contain ribosomal RNA, small RNA, and mRNA that can play a role in the pathogenesis of UPEC [65].

## **3. DISCUSSION AND CONCLUSION**

As a result of contamination with each pathotype of *E. coli*, there can be generally three clinical syndromes, including diarrhea/enteritis, urinary tract infection and septicemia/meningitis. It is important to consider that commensal strains of *E. coli* have bacterial genotoxic with a unique property of damaging the target cell DNA. Extraintestinal pathogenic *E. coli* isolated from patients with diarrhea and blood infections revealed the prevalence of toxin-producing genes. Many of the exotoxins produced by the *E. coli* strains are located in areas of chromosomal DNA called pathogenic islands, which emphasizes the potential inherent risk of this pathogenicity. Each of the exotoxins produced has unique properties. These include the following: Severe diarrhea, tissue damage and demyelination of the epithelial barrier function, cytotoxic activity, the effects of synergy with other toxins, the unknown role of some toxins in pathogenesis, the presence of plasmid toxins and the high probability of transmission of these genetic elements, manipulation of the defense

mechanism of cells hosted by toxin, infectious agent survival, and the spread of infectivity, the pathogenicity of some toxins specifically in humans and animals. Such diverse features in *E. coli* toxins show the need for more attention for various reasons. Due to the high load of *E. coli* in the body and its ability of genetic transfer, the possibility of the presence of various toxic strains are high. These strains cause systemic diseases in infants, immune deficiency patients, such as HIV patients and elderly patients, and other people with underlying illnesses. Further investigation of the *E. coli* toxins appears to be necessary. High prevalence of UPEC infection in newborns, patients and other vulnerable groups, has become an issue of concern and vital viral factors of UPEC and creation of Toxins produced by this group are the subjects of this study. Therefore, attention has been paid to this issue and numerous studies have been done to evaluate the coded toxins and their clinical significance during the course of the disease. The study of the structure of these toxins in the process of diagnosis, treatment, and even prevention is required to understand the pathogenicity of the organism. It is very important to use vaccine production in the near future.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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