ECRONICON OPEN ACCESS

MICROBIOLOGY
Review Article

Food Borne Pathogen *Escherichia coli* O157:H7 History, Sources of Transmission, Symptoms, Detection and Prevention

Osama O Ibrahim*

Consultant Biotechnology, Bio Innovation LLC, USA

***Corresponding Author:** Osama O Ibrahim, Consultant Biotechnology, Bio Innovation LLC, 7434, Korbel Dr. Gurnee IL. 60031 USA.

Received: June 24, 2015; **Published:** July 22, 2015

Abstract

Escherichia coli (*E. coli*) are bacteria that normally live in the intestines of humans and animals. Although most strains of these bacteria are harmless, several are known to produce toxins that can cause diarrhea. One particular one is *E. coli* O157:H7 that can cause severe diarrhea and kidney damage.

The bacteria are acquired by eating food containing the bacteria. The bacteria live in the intestines of some healthy cattle, and the contamination of meat may occur in the slaughtering process. Eating meat that is rare or inadequately cooked is the most common way of getting the infection. Infection can also occur after consuming foods such as lettuce, alfalfa sprouts, salami, and unpasteurized milk, juice or cider. Person-to-person transmission can occur if infected people do not wash their hands after using the bathroom. All ages can become infected with *E. coli* O157:H7, but children and the elderly are more likely to develop serious complications. Most identified cases develop severe diarrhea and abdominal cramps and blood is often seen in the stool. Usually little or no fever is present. In some patients, particularly children under five years of age, the infection can cause a complication called haemolytic uremic syndrome (HUS). A prolonged hospital stay is often required. Fortunately, most people with HUS recover completely, but it can be fatal.

Keywords: *Escherichia coli O157: H7; Verotoxin; Verotoxin receptor; Shiga toxin; Shiga toxin receptor; Shiga-like toxin; Food protection and food safety*

Abbreviations: *Vtx*: Verotoxin; *Stx*: Shiga toxin; G_3: glocotriosylceramide; HACCP: Hazard Analysis critical control Point; GMP: Good manufacture practice; PCR: Polymerase chain reaction

Introduction

Escherichia coli is a Gram negative (G-) bacteria that is commonly found in human and warm blooded animal intestines. Most *E.coli* strains are harmless and since 1890 have been used as a non pathogenic indicator for enteric pathogens, such as *Salmonella*. Currently there are six classes of *E.coli* which have been isolated that acquired virulence causing gastroenteritis to humans and are recognized to be enterovirulants of humans and animals. These enterovirulants *E.coli* are: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EaggEC), enteropathogenic (EPEC) and diffusely adherent (DAEC).

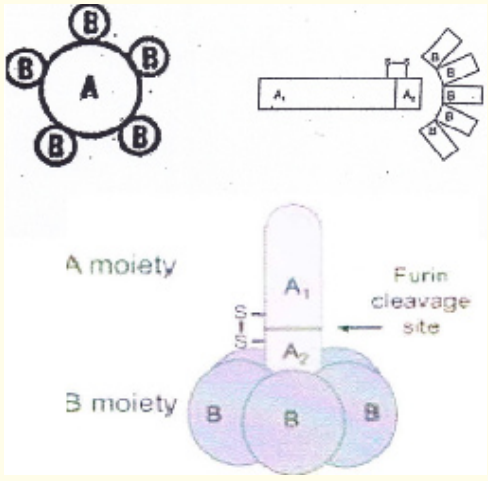
E.coli O157:H7 is within the class enterohemorrhagic (EHEC) that produces Verotoxin 1 (*Vt1*) and Verotoxin 2 (*Vt2*) these two toxins are also referred to as Shiga toxin 1(*Stx1*) and Shiga toxin 2 (*Stx2*), respectively. The ability of these strains of *E.coli* to produce Shiga toxin was acquired from a bacteriophage carrying the Shiga toxin gene from the Gram negative (G-) pathogenic bacteria *Shigella*.

History and mechanism of infection

E. coli O157:H7 is designated by its somatic O and flagella H antigens and was recognized as a human pathogen after Oregon and Michigan outbreaks in 1982. Those resulted in 47 cases of Haemolytic colitis (HC) and haemolytic uremic syndrome (HUS) of which 33 patients were hospitalized. The source of these two state outbreaks was from undercooked hamburger served by the same food restaurant chain. The food borne pathogen was isolated from patients and frozen ground beef patties were contaminated with *E.coli* O157:H7.

Shortly after *E. coli* O157:H7 was recognized to be a human pathogen, scientists observed that stool samples from children with HUS symptoms contained a substance that was toxic to Vero (African green monkey) cells [1]. This Verotoxin which is also known by the name Shiga toxin or Shiga-like toxin was produced from the isolated *E. coli* O157: H7 which caused haemolytic uremic syndrome [2].

The Shiga toxin (Figure 1) is 70 kDa proteins composed of a single 32 kDa A-subunit that associates with 5 receptor-binding 7kDa B-subunits (each) to form a ring shaped Shiga toxin [3].

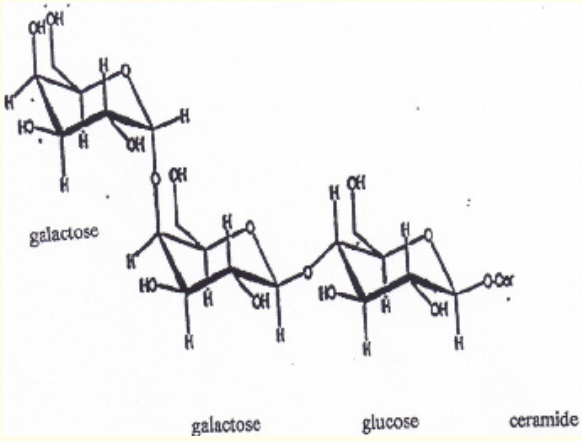


Structure of Shiga-Toxin (Stx) from E. coli O175:H7 bacteria strain [36,37]

Figure 1: *The Shiga toxin (stx) is a 70kDa oligomer that consists of one A-subunit and five B-subunits. The A-subunit contains the catalytic domain, which is active when the A_1-fragment is released upon cleavages by the intracellular protease furin. The B-subunit binds to endothelial cells surface globotriosylceramide receptors known as Gb_3receptor.*

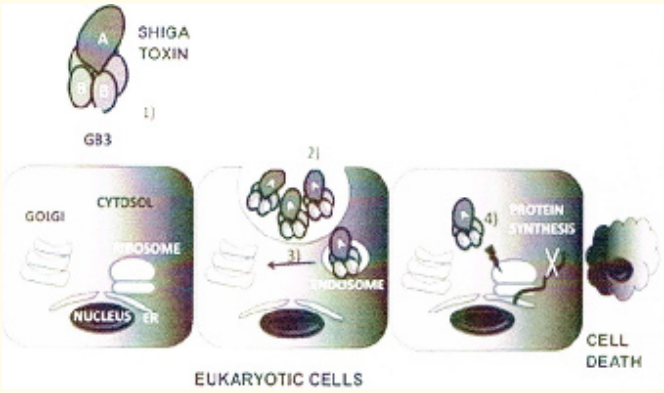
In vitro studies, Shiga toxin can damage cells after interaction with its cellular receptor glocotriosylceramide (GbOse-cer) [4] known by the name Gb_3. This receptor (Figure 2) has been found in human kidney endothelial cells and in cultured endothelial cells [5].

The B-subunits of Shiga toxin are responsible for binding to the Gb_3 receptor on endothelial cells. The bound Shiga toxin is endocytosed inside the endothelial cell and transported to the *trans-Golgi* network where the enzyme Furine [6] is located. Furine is a calcium-dependent serine endo-protease enzyme primarily located in the *trans-Golgi* networks [7]. Furine cleaves the A-subunit of Shiga toxin yielding A1 (27.5kDa) and A2 (4.5kDa) fragments [8]. The A_1 fragment that has *N*-glycosidase enzymatic activity translocates to cytosol and inhibits protein synthesis. The specific *N*-glycosidase enzyme cleaves off a single adenine residue in position 4323 from the 5' terminus of 28S rRNA of the 60S ribosome subunit [9]. This cleavage inhibits binding of the elongation factor-1 (EF-1)/aminocyl-tRNA complex to the ribosome, resulting in the inhibition of protein synthesis (Figure 3). It is widely assumed that inhibition of protein syntheses is an event that triggers apoptosis in kidney endothelial cells and consequently the development of haemolytic uremic syndrome [10] (HUS).



Chemical structure of Shiga-toxin (Stx)receptor [36] (Gb_3)

Figure 2: *The B-subunit of Shiga toxin binds to endothelial cells via galactose-containing glycolipids known as the globotrisylceramide receptor, also known by the name Gb_3 receptor.*



Shiga-toxin (Stx) mechanism of action[37]

Figure 3: *The following are the five steps of the Shiga-toxin mechanism of action inside human endothelial cells: 1) Shiga toxin (Stx) is internalized by endocytosis. 2) Shiga toxin undergoes retrograde transport to the trans-Golgi network (TGN). 3) Transported to the endoplasmic reticulum (ER). 4) In the endoplasmic reticulum (ER), Shiga toxin encounters its target, the ribosome andinactivates it. 5) As a consequence, Shiga toxin inhibits protein synthesis, causing cell death by apoptosis, thus, causing hemolytic uremic syndrome (HUS).*

Haemolytic uremic syndrome symptoms

Haemolytic uremic syndrome (HUS) is characterized by acute renal failure, haemolytic anemia and thrombocytopenia. The symptoms start with abdominal cramps and mild non-bloody diarrhea that in some cases progresses to bloody diarrhea which is the symptom for hemorrhagic colitis (HC). Fever and vomiting may also occur [11]. The incubation period after eating food contaminated with *E.coli*

O157:H7can range from 3-8 days. Most patients recover within 10 days, but in a small portion of patients particularly children and the elderly can develop haemolytic uremic syndrome that may lead to death. Individuals who experience bloody diarrhea or severe abdominal cramps should seek medical care. Other HUS-associated complications may include seizures, coma, stroke, colonic perforation, pancreatitis, and hypertension. It is estimated that 15% of cases lead to early development of chronic kidney failure [12].

HUS patient require dialysis and the mortality rate is 3-5%. Antibiotics are not part of treatment and possibly increase the risk of subsequent haemolytic uremic syndrome symptoms. Scientists still do not know how the best treatment for this infection. Certain novel treatment strategies such as using anti-Verotoxin (anti-Shiga toxin) antibodies have been proposed.

In the United States this pathogen results in an estimated 2,100 hospitalization annually and patients developed HUS often require prolonged hospitalization, and long-term follow-up that is costly for both patients and Health insurance organizations.

Source of infection and transmission

Under cooked or raw hamburger (ground beef) has been the most common food associated with the outbreaks [13] that occurred in 1982, 1992 and 1993 in United States and affected more than 500 people [14]. The largest reported *E. coli* O157:H7 outbreaks, which caused thousands of illnesses, occurred in Japan in 1996 and 1997. These two outbreaks in Japan were associated with radish sprouts and alfalfa sprouts. Alfalfa sprouts were also implicated in a recent outbreak in the United States. Other foods that have been associated with *E.coli* O157:H7 outbreaks worldwide are unpasteurized fruit juice, cider, raw milk, dry-cured salami, lettuce, game meat and cheese curd [15].

Since 1982 *E.coli* O157:H7 has been implicated in outbreaks worldwide and is the primary cause of haemolytic colitis and haemolytic uremic syndrome in the United States, Canada, Great Britain, other regions in Europe, Japan , other regions of Asia and in other parts of the world [16].

Ingestion of a low infectious dose fewer than 10-100 CFU of *E.coli* O157:H7 in contaminated food, water or oral contact with contaminated surfaces is sufficient to cause infection. This infectious dose is very low compared to the infectious dose of over one-million CFU for other pathogenic *E.coli* strains. It has been suggested that *E.coli* O157:H7 strains in these out breaks may have enhanced acid tolerance [17].

The reservoir of this *E.coli* O157:H7 appears to be mainly cattle. In addition, other ruminants such as sheep, goats, and deer are considered significant reservoirs as well. Other animals (pigs, horses, rabbits, dogs, and cats) and birds (chickens and turkeys) have been occasionally found infected. Scientist still does not know how cattle, which are the main source of infection for humans, become infected. Cattle lack the Shiga toxin receptor (G_3) and therefore, can be asymptomatic carriers or super-shedders of the bacterium. Super-shedders are defined as cattle exhibiting rectal-anal junction colonization with *E.coli* O157:H7 excreting >103to4 CFU per gram of faces and account for > 90 % of all *E.coli* O157:H7 excreted [18].

Physiological Properties of *E. coli* O157:H7

Like other microorganisms the survival and growth of *E. coli* O157:H7 in foods are dependent on the interaction of various intrinsic and extrinsic factors such as temperature, PH, and water activity.

Temperature: *E.coli* O157:H7 grow at room temperature with optimum temperature at approximately 45°C (dependent on the strain) and the minimum temperature is approximately 8-10°C [19]. This lower temperature indicates that these pathogenic bacteria can survive and grow in refrigerated foods.

PH: The optimum PH of *E.coli* O157:H7 is in the range of 5.5-7.5. This optimum pH is similar to non pathogenic *E.coli*. Some strains of *E.coli* O157:H7 are acid tolerant and can survive for several weeks or months in a variety of acidic foods [20]. The survival in acidic foods is an important phenomenon, since several outbreaks have been associated with acidic foods and beverages such as fermented sausages, apple cider, and apple juice.

Water Activity: Studies of the effect of water activities on the survival and growth of *E.coli* O157:H7 are focused on the effect of sodium chloride for preservation. *E. coli* O157: H7 behaves similarly to non pathogenic *E.coli* with a minimum water activity (a_w) at 0.98 [21].

E.coli O157:H7 can be eradicated in processed foods by food preservation methods such as thermal kill, antimicrobial peptides, chemical preservatives and by other bio or physical preservation methods.

Isolation and Identification of *E.coli* O157: H7

Since the detection of pathogenic *E.coli* is based on its virulence properties, detection methods in food products, fresh produce and water require isolation and identification of the target organism using standard procedures for enrichments and isolation [22] before testing for identification of the specific virulence traits.

E.coli O157:H7 is phenotypically distinct from other *E. coli*. It exhibits slowly or no sorbitol fermentation [23] and does not have B-glucuronidase enzymatic activity. These two phenotypic traits are often used to isolate the pathogenic bacteria *E. coli* O157:H7. Sorbitol - MacConky (SMAC) agar was developed to take advantage of this characteristic by substituting the carbohydrate sorbitol for lactose in MacConky agar. SMAC agar is the medium of choice for isolation of *E. coli* O157:H7 [24]. The sorbitol negative colonies isolated from SMAC agar plates are the subject of additional identification methods.

Identification methods require two methods of testing (presumptive and conformational tests). Both tests utilize microbial analytical methods such as biochemical, serotyping for O157 and H7 antigens and polymerase chain reaction [25] (PCR) for Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) genes to confirm the toxigenic potential of the isolated *E.coli*.

Some laboratories take advantage of the fact that *E.coli* O157:H7 does not have B-glucuronidase enzymatic activity by using broth or agar medium containing the substrate 4-methyl-umbellifery-B-D-glucuronide (MUG) [26]. When MUG is cleared by the B-glucuronides, a florescent product is produced that is detectable with long-wave ultraviolet light. *E.coli* O157:H7 that produce Shiga toxins lack the enzyme and are MUG negative.

The secretion of the two Shiga toxins (*STX1*) and (*STX2*) by *E.coli* O157:H7 can also be utilized in cytotoxicity assays on Vero or Hela cells in the confirmation tests [27].

Also, there are commercially available ELISA kits [28], Gene probes and PCR assays [29] specific for *Stx1* and *Stx2* toxins, plus there are other trait markers also available for *E.coli* O157:H7 for confirmation tests.

Contamination prevention with *E.coli* O157:H7

Because food or water contaminated with *E.coli* O157:H7 can cause serious illness, it is critical to reduce this risk of contamination from farms, slaughterhouses, food manufacturers, and consumers handling.

The food Industry is responsible for producing safe foods that meet federal standards under the *Food and Drug Acts, Meat Inspection Acts* and other related regulations [30] (HCCP and GMP).

Hazard Analysis and Critical Control Points [31]

Hazard Analysis and Critical Control Points (HACCP) is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, for manufacturing, distribution and consumption of the finished product.

It is a system for the identification, evaluation and control of food safety hazards based on seven principles:

a. Conducting a hazard analysis.
b. Determining the critical control points (CCPs).
c. Establishing the critical limits.
d. Establishing monitoring procedures.

e. Establishing corrective actions.
f. Establishing verification procedures.
g. Establishing record-keeping and documentation procedures.

These seven principles of HACCP have been accepted by government agencies, trade associations and the food industry. Currently, food safety systems based on the HACCP principles have been successfully applied in food processing plants, retail food stores, and food service operations worldwide.

Consumers can also implement HACCP-like practices in the home by following proper storage, handling, cooking and cleaning procedures, from the time of purchasing meat, poultry, and fresh produce (vegetables and fruits) to the time of serving a meal. There are many steps to be taken by the consumer to prevent bacterial growth and to ensure food safety. These steps for example are: properly refrigerating foods, thoroughly cooking meat and poultry, keeping raw meat and poultry separated from cooked foods and from fresh ready-to-eat foods.

Good Manufacturing Practice [32]

Good Manufacturing Practice (GMP) guidelines are an essential foundation for the development and implementation of successful HACCP plans. These guidelines provide guidance for manufacturing, testing, and quality assurance in order to assist in reducing the risk of food borne illness and to secure the production and distribution of safe food for human consumption. Many countries follow GMP procedures and have created their own GMP guidelines which corresponded to their legislation.

All guidelines follow these basic principles:

1. Maintain a clean and hygienic manufacturing area.
2. Control environmental conditions in order to prevent cross contamination of food products from other in-process foods.
3. Develop manufacturing processes that are clearly defined and controlled.
4. Validate all critical control points (CCPs) to insure safety and constancy in compliance to the established specifications.
5. Control manufacturing processes, and evaluate and validate any changes in the processes.
6. Write instructions and procedures clearly (Good Documentation Practice) and record process data during manufacturing.
7. Minimize the risk of contamination during the distribution of products.
8. Establish a system for quick recall of any unsafe product from sale or supply.

Finally, food safety is a serious matter and both the HACCP Plan and GMP guidelines are the safeguards for manufacturing and distribution of safe and healthy foods to consumers.

Summary

The purpose of this review is to provide important aspects of the contamination of foods, meat products and fresh produce with *Escherichia coli* O157:H7.

Escherichia coli are one of the bacteria from the family of Enterobacteriaceae usually found in the digestive system of both healthy humans and animals and are usually harmless to the host. It is a facultative anaerobe, gram negative (G-) bacterium.

There are six groups of pathogenic *E.coli* which has emerged that causes enteric diseases including diarrhea. These six groups are classified based on their virulence factors. Four of these six groups have been implicated in food or water borne illnesses [33]. These four are:

1) Enterotoxigenic *E. coli* (ETEC) which commonly occurs in under-developed countries and is recognized as the causative agent of traveler diarrhea with little or no fever.
2) Enteropathogenic *E.coli* (EPEC) causes a profuse watery diarrhea and is the leading cause of infantile diarrhea in developing countries.

3) Enteroinvasive *E.coli* (EIEC) is closely related to the bacteria *Schigella* that infect only humansand cause an invasive dysenteric form of diarrhea.
4) Enterohemorrhagic *E.coli* (EHEC) causes bloody diarrhea hemorrhagic colitis [11] (HC), which can progress to potentially fatal haemolytic uremic syndrome (HUS).

Enter hemorrhagic *E.coli* (EHEC) are typified by the secretion of two classes of verotoxins (1 and 2) which are also known by the name Shiga toxins (*Stx-1*) and (*Stx-2*). One of these EHEC prototypes is *E. coli* O157:H7, which is the most implicated in illness worldwide. The infectious dose for *E. coli* O157: H7 is estimated to be 10-100 cells, mostly in foods but also water borne. It has been implicated for causing fatal haemolytic uremic syndrome in humans after the ingestion of undercooked beef, raw milk, cold sandwiches, unpasteurized apple juice, unpasteurized milk, fruits, vegetables, and sprouts.

In the United States, the Center for Disease Control (CDC) has estimated that food borne *E. coli* O157:H7 infections cause 73,000 illnesses, 2,200 hospitalization and 60 deaths annually [34].

It is clear that microbiologists, molecular biologists and food scientists have made great research efforts in the understanding, detection and prevention of *E. coli* O157:H7 and related EHEC in food. Their efforts are integrated as part of food safety programs that have assisted in minimizing the impact of this immerging food borne pathogen threat to human health. However, large outbreaks and sporadic cases continue to occur.

It is estimated the average annual cost from this food borne illness is 405 million dollars, including lost productivity, medical care, and premature death [35].

Bibliography

1. O'Brien AD and LaVeck GD. "Immunochemical and cytotoxic activities of Schigella dysenteriae-1 (Shiga) and Shiga-like toxin". *Infection and Immunity* 35 (1982): 1151-1154.
2. Nield GH. "Hemolytic Uremic Syndrome". *Nephron* 59 (1991): 194-198.
3. O'Brien AD and LaVeck GD. "Purification and characterization of a Schigella dysenteriae-1 (Shiga) toxin purified by anti-Shiga affinity chromatography". *Infection and Immunity* 40 (1983): 675-683.
4. Lingwood CA., *et al.* "Glycosylated binding of purified and recombinant Escherichia coli produced verotoxin *in vitro*". *The Journal of Biological Chemistry* 262 (1987): 8834-8839.
5. Obrig TG., *et al*. "Endothelia heterogeneity in Shiga toxin receptors and response". *The Journal of Biological Chemistry* 268 (1993): 15484-15488.
6. Halban PA and Irmenger JC. "Sorting and processing secretory proteins". *Biochemical Journal* 299 (1944): 1-18.
7. Bosshart H., *et al*. "The cytoplasmic domain mediates localization of furin to the trans-Golgi network in route to endothelial/lysosomal system". *Proceedings of the National Academy of Sciences* 84 (1987): 4364-4368.
8. Olsnes S., *et al*. "Subunit structure of Schigella cytotoxin". *The Journal of Biological Chemistry* 256 (1981): 8732-8738.
9. Saxina SK., *et al*. "Shiga toxin, Shiga-like toxin II variant, and ricin are single-site RNA N-glycosidase of 28 S RNA when microinjected into Xenopus oocytes". *The Journal of Biological Chemistry* 264 (1989): 596-601.
10. William JM., *et al*. "Comparison of ribosome-inactivating proteins in induction of apoptosis". *Toxicology Letters* 91 (1997): 121-127.
11. Tarr PI. "Escherichia coli O1575:H7 Clinical diagnostic and epidemiological aspects of human infection". *Clinical Infectious Diseases* 20 (1995): 1-10.
12. Siegler RL., *et al*. "Recurrent hemolytic uremic syndrome secondary to Escherichia coli O157:H7 infection". Pediatrics 91 (1993): 666-668.

13. Griffin PM and Tsuxe RV. "The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterhemorrhagic *E. coli* and the associated hemolytic uremic syndrome". *Epidemiologic Reviews* 13 (1991): 60-98.
14. CDC. "Update; Multistate outbreak of Escherichia O157:H7 infections from hamburgers-western United States, Centers of Disease Control and Prevention Atlanta, Ga". *Mortal Weekly Report* 44 (1993): 550-551.
15. M Ellin Doyle., *et al*. "Human illness caused by *E.coli* O157:H7 from foods and non-foods sources". *Food Research Institute-UM-Madison* (2006).
16. Berger SA. "*E.coli* Diarrhoea Global status". (2011).
17. Buchanan RL and Edelson GS. "Culturing enterhemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells". *Applied and Environmental Microbiology* 62 (1996): 4009-4013.
18. F Omisakin., *et al*. "Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter". *Applied and Environmental Microbiology* 69 (2003): 2444-2447.
19. Rajkowski KT and Marmer BS. "Growth of *Escherichia coli* O157:H7 at fluctuating incubation temperatures". *Journal of Food Protection* 58 (1995): 1307-1313.
20. Zhao T., *et al*. "Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider without preservatives". *Applied and Environmental Microbiology* 59 (1993): 2526-2530.
21. Buchanan RL and Bagi LK. "Effect of water activity and humectant identify on the growth kinetics of *Escherichia coli* O157: H 7". Food Microbiology 14 (1997): 413-423.
22. Weagant SD., *et al*. "An Improved rapid technique for isolation of *E. coli* O157:H7 from foods". *Journal of food protection* 58 (1995): 7-12.
23. Fratamico PM., *et al*. "Virulence of an *Escherichia coli* O157:H7 sorbitol-positive mutant". *Applied and Environmental Microbiology* 59 (1993): 4245-4252.
24. Novicki TJ., *et al*. "Comparison of sorbitol MacConkey agar and a two-step method which utilizes enzyme-linked immunosorbent assay toxin testing and a chromogenic agar to detect and isolate enterohemorrhagic *Escherichia coli*". *Journal of Clinical Microbiology* 38.2 (2000): 547-551.
25. Feng P and SR Monday. "Multiplex PCR for detection of trait and virulence factors in enterhemorrhaic *Escherichia coli* sterotype". *BBA Molecular Cell Research* 14 (2000): 333-337.
26. Lloyd J Moberg. "Fluorogenic assay for rapid detection of *Escherichia coli* in food". *Applied and Environmental Microbiology* (1985): 1383-1387.
27. Said Bouzari., *et al*. "Study of induction of apoptosis on Hela and vero cells by recombinant Shiga toxin and its subunits". *Cytotechnology* 60.1-3 (2009): 105-113.
28. Nisha V Padhye and Michael P Doyle. "Rapid procedure for detection Enterohemorrhagic *Escherichia coli* O157:H7 in food". *Applied and Environmental Microbiology* (1991): 2693-2698.
29. Narayanan Jothikumar and Mansel W. "Griffiths, Rapid detection of *Escherichia coli* O157:H7 with Multiplex Real-Time PCR assays". *Applied and Environmental Microbiology* (2002): 3169-3171.
30. Dunkelberger Edward. "The statutory basis for the FDA's food safety assurance programs: From GMP, to emergency permits control, to HACCP". *Food and Drug Law Journal* 50 (1995): 357-383.
31. FDA 2001 Food Code-Annex5: HACCP Guidelines. *U.S. Department of Health and Human services. Public Health Service. Food and Drug Administration* (Updated April 2004).
32. GMPs-Section One: Current Food Good manufacturing Practice. *Food and Drug Administration* (August 9, 2004).
33. Todar K. "Pathogenic *E. coli*". *Online Textbook of Bacteriology*. University of Wisconsin–Madison Department of Bacteriology. (2007): 11-30.
34. CDC Estimate: Finding; CDC estimates of food borne illness in the United States (2011).
35. Frenzen Paul D. "An online cost calculator for estimating the economic cost of illness due to Shiga toxin-producing *E. coli* (STEC) 0157 infections". *Economic information bulletin* 28 (2007).

36. Osama O Ibrahim. "Target angiogenesis with Shiga-like toxin fused to vascular endothelial growth factor". *PhD Dissertation New York Medical College* (2001).
37. Alline R., *et al*. "Shiga toxin in enterohemorrhagic *E. coli*: Regulation and novel anti-virulence strategies". *Frontiers in Cellular and Infection Microbiology* (2011).

Volume 2 Issue 1 July 2015