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Escherichia coli and Food Safety

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Abstract

Foodborne pathogens are evaluated as an important risk factor in terms of public health in developed and developing countries due to their extensiveness all around the world. *Escherichia coli* and other coliform bacteria are important foodborne pathogens. Some of the most important sources of contamination for these groups of microorganisms are reported as: areas with unfavorable hygiene, contaminated waste water, meat products, cereal products and vegetables. Total coliform bacteria and *E. coli* count is known to be the indicator of unfavorable hygienic conditions and fecal contamination in foods. Foodborne diseases, however, are a global issue. A joint approach by all countries and related international organizations is a prerequisite for detection and control of foodborne problems that pose a threat to human health and international trade. Despite their complicated biology, epidemiology and analyses, most foodborne diseases are preventable. It is of vital importance for public health that consumers and food producers act in accordance with the principles regarding internationally accepted safety methods.

Keywords: *Escherichia coli*, food safety, foodborne diseases

1. Introduction

Microorganism of varying types and numbers can be found on food of animal and plant origin. The types and number of microorganism on food can be changed due to food processing, inappropriate purchasing, storing, preparing, cooking or serving. Increase in the number of these microorganisms due to the abovementioned changes may lead to spoiling of the food, causing a pathogenic effect on humans. The most important of foodborne pathogenic bacteria is *Escherichia coli*. It is transmitted through fecal or oral route and it should, under no circumstances, be present in any food. The most prominent symptom caused by this microorganism is its diarrheagenic effect. Moreover, it is known to cause sepsis, meningitis and many enteric diseases. Inability to ensure food safety is one of the biggest food-related problems. Food safety means ensuring necessary hygienic conditions and taking protective safety precautions for a healthy and safe food production throughout all processes from obtaining raw materials to production, transportation, storage, distribution and consumption of food. This section will focus on the pathogenic characteristics of food contaminated with *E. coli*, food contamination cases, current food safety approaches and methods of prevention/protection.

2. *Escherichia coli* and food poisoning

Escherichia coli, one of the 30 members of the bacterial family of *Enterobacteriaceae*, is a coliform bacterium and is one of the 6 types of *Escherichia* species (*E. adecaroxylate*, *E. blattae*, *E. fergusonii*, *E. hermannii* and *E. vulneris*). It is a gram-negative, non-spore-forming, facultative, anaerobic, rod shaped, mesophilic bacterium that grows in 7–45°C. The group of coliform bacteria consists of *Citrobacter*, *Enterobacter*, *Klebsiella* and *Escherichia*. While there are bacteria of fecal origin among coliform bacteria, there are also bacteria of plant origin such as *Enterobacter aerogenes*, *Citrobacter freundii*, and *Klebsiella pneumoniae*. Presence of coliform group in food is indicative of fecal contamination, poor hygienic conditions or existence of enteric pathogens. For instance, the presence of coliform bacteria in raw milk is an indication of poor hygiene in milking or storage conditions. The presence of coliform bacteria in raw or frozen fruits and vegetables is not important as *Enterobacter*, *Citrobacter* and *Klebsiella* are naturally present in the microbiota of plants. However, *E. coli* presence in fruits and vegetables is very important in terms of inadequate hygiene. *E. coli* is an important pathogen as it is an indicator of fecal contamination in foods and drinking water. Due to this characteristic, it is considered as an indicator bacterium in food safety and hygiene [1–3].

Being the prominent bacterium in the facultative anaerobic microbiota of the intestines, *E. coli* is widespread in stool and the environment. Some of its pathogenic strains both cause intoxication by creating toxins and cause gastroenteritis, pathologic kidney and brain damage by causing infection-type food poisoning through cellular increase. Some enterotoxin producing *E. coli* strains are divided into two groups as heat-stable and heat-labile. The heat-stable toxin is known as stable toxin (ST) and the heat-labile toxin is known as labile toxin (LT). Both toxins can be found together or separately. Moreover, pathogenic strains are also known to cause serious diseases such as diarrhea, peritonitis, mastitis, septicemia, pneumonia and neonatal meningitis. Among gram-negative bacilli, *E. coli* is the most widespread pathogen that causes meningitis especially in neonatal period. It has serious morbidity and mortality rates worldwide. The mortality rates in neonatal meningitis cases are reported to vary between 15–40% and 50% of the survivors are reported to continue their lives with neurological damage [2, 4]. Intestinal pathogenic *E. coli* are classified as shown in **Figure 1** at least six subgroups/pathotypes as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely-adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC). EHEC is also known as Shiga toxin (stx) producing *E. coli* (STEC) and verotoxin producing *E. coli* (VTEC) [5, 6].

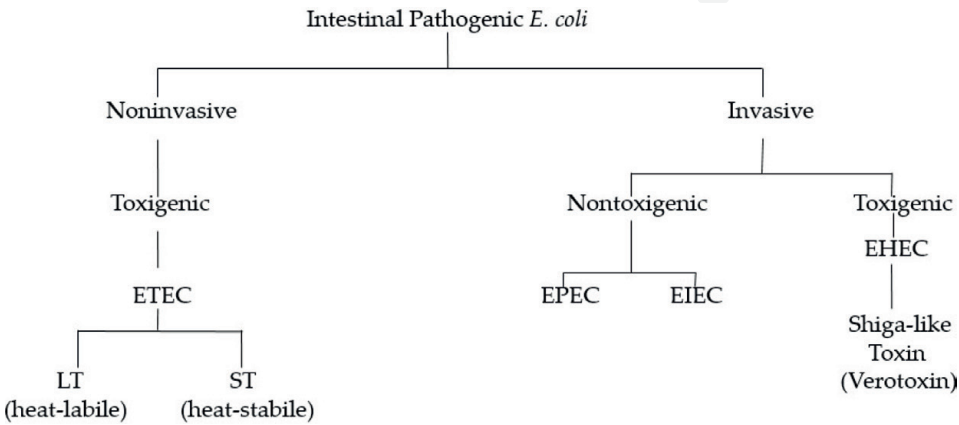


Figure 1.
Mechanism of intestinal pathogenic *E. coli* strains [6].

2.1 Enterotoxigenic *E. coli* (ETEC)

People living in developing countries have often been reported to have this pathotype in their feces and shown to have developed immunity against this microorganism. Being a cause of mortality in children under 5, the most frequently observed microorganism in childhood diarrhea is ETEC and it is also responsible for 30–60% of travelers' diarrhea. Infection is characterized by watery diarrhea and, depending on the person, its course may range from a normal course to cholera-like defecation with the addition of symptoms such as vomiting and high fever [2, 4, 7]. Diarrhea is the most common causes of mortality in society and among young children, especially those living in Asia and sub-Saharan Africa with inadequate health-care systems and limited access to clean drinking water. Recent systematic studies have reported that each year an estimated 600,000 children under the age of 5 lose their lives. Diarrhea occurs due to the consumption of food or water contaminated with viral, bacterial or parasitic pathogens. Among these potential pathogens, the most common cause of diarrhea in children under five is the ETEC (heat-stable – ST and/or heat-labile – LT type toxin) producing *E. coli* strains. Through the production of fimbrial or non-fimbrial adhesins, ETEC strains cause hypersecretion of fluids by producing enterotoxins that disrupts fluid and electrolyte homeostasis in the epithelial cells of small intestines, leading to watery diarrhea. Without rehydration, moderate or severe diarrhea could lead to dehydration and acute mortality [7].

2.2 Enteropathogenic *E. coli* (EPEC)

It is known to be the oldest *E. coli* serotype causing diarrhea and its most important characteristic is adherence. In EPEC infections, vomiting and low body temperature are observed in addition to watery diarrhea [5]. It is known to cause diarrhea in infants and outbreaks can occur in neonatal care units. Humans, pigs and bovines may be infected with this microorganism. EPEC is transmitted from person to person, however; rarely, it is also known to spread through contaminated food and water [4, 6].

The ability to produce attaching and effacing (A/E) lesions is a distinctive phenotype for EPEC. Bacteria cause extensive deterioration on microvilli by strongly adhering to the host cell membrane. This adherence to the cell is mediated by an outer membrane protein called intimin. Moreover, depending on the presence of *E. coli* adherence factor – EAF, EPEC is classified as typical EPEC (tEPEC) and atypical (aEPEC) strains. In addition, as a distinctive factor, all EPEC strains lack the Shiga toxin (*stx*) producing genes. Among single-pathogen infections, EPEC has the second highest severity score after rotavirus, followed by ETEC. Diarrheagenic *E. coli*, especially EPEC, ETEC and EAEC are found out to be the main pathogens related to chronic diarrhea and its complications that lasts more than 14 days in developing countries. Moreover, among children with chronic diarrhea in developing countries, aEPEC was the most common pathogen isolated and it is the most common clinical case. These findings show that aEPEC may have a tendency to be naturally more chronic than other diarrheagenic *E. coli* [8, 9].

2.3 Enteroaggregative *E. coli* (EAEC)

This pathotype is a foodborne enteropathogen observed in acute and persistent diarrhea cases in children, patients with suppressed immune systems in developing countries and people traveling to endemic regions. Growth disorders and cognitive disorders in children living in developing countries, stem from EAEC infections. In the pathogenesis of EAEC, the first step is the strong adherence to the intestinal

mucosa. The second step is leading to the development of enterotoxins and cytotoxins and the third step is known to be characterized with the ability to induce mucosal inflammation. Many different virulence factors regarding these three steps have been defined, however; none of them are present in all strains. Three adherence models related to EAEC have been defined. In addition to the localized adherence (LA) model that was defined first, there is also a diffuse adherence (DA) model and aggregative adherence (AA) model. The strains corresponding to the AA pattern were later defined as “Enteroadherent-aggregative *E. coli*”. However, this term was then replaced with the current name “Enteroaggregative *E. coli*”. AA phenotype has to be present in order for an *E. coli* strain of EAEC pathotype to be classified [10].

It is commonly found in foods in Mexico, including desserts and salsa sauces, and the visitors of the country are known to be more sensitive to EAEC infections during their stay rather than ETEC, which they are the most susceptible to. The reason behind this is the EAEC’s ability to suppress the immune system and cause chronic infection. EAEC is also more resistant to antibiotics compared to the other diarrheagenic pathogens. Persistent infection and chronic disruption in intestinal functions cause malnutrition and decline in physical and mental development, especially in children. Malnutrition, which is observed due to micronutrient deficiency, induces infection. Development of infection induces malnutrition. This whole cycle increases the burden of acute diarrhea [11].

2.4 Diffusely-adherent *E. coli* (DAEC)

Hep-2 or HeLa cell cultures are called DAEC due to their diffuse adherence characteristics. DAEC serotypes are known to cause chronic diarrhea in children between the ages of 1 and 5. They cause degradation in the intestinal epithelium by binding to proteins that accelerate degradation. Mild diarrhea void of fecal leukocytes is the indication of infection. In France, DAEC strains were found out to be widespread in diarrhea cases observed in inpatients from a hospital with no other enteropathogen. This situation indicates that DAEC strains may be an important diarrheagenic pathogen in developed countries. Recent studies show that some DAEC strains contain virulence factors present in uropathogenic *E. coli* (UPEC) strains [5, 12].

2.5 Enteroinvasive *E. coli* (EIEC)

EIEC strains causing inflammatory damage in intestinal mucosa and submucosa are very similar to those produced by *Shigella*. These microorganisms have the same spreading and reproducing abilities inside epithelial cells. However, clinically, EIEC-related watery diarrhea is much more commonly observed than dysentery caused by *Shigella*. O antigens of EIEC can cross-react with O antigens of *Shigella*. The disease starts with severe abdominal cramping, weakness, watery stool, difficulty urinating and fever. It could rarely aggravate and turn into watery stool containing blood or mucus. The fecal leukocytes observed in shigellosis may also be observed in the mucus smear of a person infected with EIEC. EIEC infections are endemic to less developed countries and are reported to be rarely observed infections in developed countries. The incubation period is observed as 10–18 hours. There is evidence showing that EIEC is transmitted through contaminated foods. Just like in shigellosis, cases of diarrhea with enteroinvasive strains can be treated by using antimicrobials effective against *Shigella* isolates [13]. In a study conducted to investigate the effects of antibiotic usage, stool samples were analyzed to find out whether it affected pathogen findings. Four and fifty-six tourists from Finland were all informed about antibiotic usage during travel and stool samples were collected from them both before and after the travel. There were differences between the

travelers that visited various countries before and the ones that did not use any antibiotics in terms of *Enterobacteriaceae* findings, as well as some health problems during the travel and pathogenic findings in stools [14].

2.6 Enterohemorrhagic *E. coli* (EHEC)

EHEC are also named Shiga toxin producing *E. coli* (STEC) and also verotoxin producing *E. coli* (VTEC). All strains of EHEC produce Shiga toxins that destroys vero cells similarly to Shiga toxins produced by *Shigella*. *E. coli* O157: H7, first defined after the outbreak associated with the consumption of rare cooked minced meat in 1982, is the primary cause of EHEC infection in industrialized countries including the USA, Canada and England. O26, O103, O111 and O145 can be listed among the other EHEC serogroups responsible for foodborne diseases. Even though the O157 strains are the ones that draw the most attention, the strains of other EHEC serogroups, especially O111, are gradually getting reported more and more around the world. Based on the severity of the disease, EHEC is regarded as the most serious *E. coli* strain among foodborne pathogens. *E. coli* O157:H7, differ from the other *E. coli* serotypes because of some of its characteristics, which are: not being able to grow in or above 42°C, not being able to ferment sorbitol, not having β -glucuronidase enzymes and producing enterohemolysins. Shiga-like toxin produced by *E. coli* O157:H7 is cytotoxic for human colon and duodenum. This toxin causes accumulation of fluid in intestines and lesions in colon through destruction of crypt epithelia. Intimin makes adhesion to the intestinal canal easier [5, 15].

EHEC has a wide spectrum including watery or bloody diarrhea and hemolytic uremic syndrome (HUS), which is an important factor in acute renal failure in children. The biggest EHEC O104:H4 outbreak was in Germany in 2011 with 855 HUS cases in 3842 people and 53 mortalities. This incidence, which raised concern all around the world, shows the importance of EHEC in terms of human health. Bovines are the main reservoir for these microorganisms to live on asymptotically for years. Other smaller reservoirs for these microorganisms include sheep, goats, dogs, pigs and poultry. Other places where EHEC could stay alive for months include; bovine feces, soil and water. Butchering or processing of animals or contamination of plants through contaminated water or manure are the main routes for EHEC to spread to the food chain [16]. Following 3–12 days of incubation period after infection with *E. coli* O157:H7, watery diarrhea is observed as well as abdominal cramps and pain. In some cases, hemorrhagic colitis (HC) which is also known as bloody diarrhea, thrombotic thrombocytopenic purpura (TTP), fever and vomiting are included in the important clinical findings to be observed. Most patients recover within 10 days, however; depending on the serotype of the EHEC strain and *stx* subtype, HUS may develop 1 week after the start of diarrhea, that may lead to mortality especially in children and elderly people. HUS is characterized with acute renal failure, hemolytic anemia and thrombocytopenia. Coma, stroke, colon perforation, pancreatitis and hypertension are included among the other complications of HUS. It is estimated to lead to the early development of chronic renal failure in 15% of cases. Dialysis is necessary for HUS patients and mortality rate is 35%. Moreover, it is more commonly observed in women (70%) and during pregnancy (13%). Good treatment for this infection is still lacking, however; some new treatment strategies such as the usage of anti-vero toxin (anti-Shigatoxin) antibodies have been suggested. TTP, on the other hand, is clinically similar to HUS and fever, abdominal pain, gastrointestinal hemorrhage and central nervous system disorders are listed among complications that may develop. Frequently, it forms blood clots in the brain and result in mortality [2, 15–20].

The incidence and epidemiology of the important serotypes of *E. coli* are given in **Table 1**.

Pathogenic <i>E. coli</i>	Site of infection	Associated disease	Incidence	Target population	Significant transmission route
ETEC	Small intestine	Traveler's diarrhea, chronic childhood diarrhea (in developing countries)	16 U.S. outbreaks (1996–2003); prevalence 1.4% in patients with diarrhea; 79,420 cases of travelers' diarrhea each year (in the USA)	International travelers and children in developing countries	Food (raw produce, street vendors) and water
EPEC	Small intestine	Infant diarrhea	Hundreds of thousands of deaths world wide	Children in developing countries	Water, infant formula
EHEC	Large intestine	Hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS)	110,000 cases and 61 deaths annually in the USA	All ages	Food (beef produce), person-to-person, water, animals
EIEC	Large intestine	Dysentery	Low in developed countries	Children in developing countries	Water (rare), person-to-person
EAEC	Intestine	Watery diarrhea with or without blood in the stool, acute and chronic	Developed and developing countries	Children and adults, travelers	Food, water, person-to-person

Table 1.
Summary of incidence and epidemiology of E. coli serotype [6].

3. Food safety and high-risk foods

Food safety means ensuring consumer safety and protecting products from biological, physical and chemical hazards throughout the whole process starting from the field to processing, storing, distributing, preparing and cooking [21]. In many countries around the world, people started to have a more conscious perspective on food and environment. Consumers tend to prefer food that is more natural, less processed, environment-friendly, healthy and produced safely. This tendency makes up the basis of the “preventive/protective” (pro-active) approach for measurements to be taken towards food safety both nationally and globally. This approach based on risk analysis is the most appropriate and effective method for controlling foodborne hazards. It also necessitates the application of proper control systems in the production chain [22]. Foodborne diseases are a global subject. A common approach by all countries and related international organizations is a prerequisite for the detection and control of foodborne problems threatening human health and international trade. Despite their complicated biology, epidemiology and analyses, most foodborne diseases are preventable. Public health institutions, food industry and consumers must be devoted to prevent foods from getting

contaminated at farms, restaurants and homes. In outbreaks of foodborne diseases, continuous monitoring is vital for revealing the disease tendencies in foods, regions and associated pathogens. Genotype and subtype information obtained from contaminated strains are required for tracing the source of contamination, characterizing and comparing the strains [23].

The food safety management systems with a classical basis that were once accepted for safe production and consumption of foods has proven to be inefficient and researchers/organizations proposed the “risk-based food safety” approach. Risk-based food safety approach is significantly different than the classical hazard-based approach. In this regard, a food safety management system aims at estimating the risks to human health as well as defining, choosing and implementing strategies to control and decrease these risks. According to Codex Alimentarius, risk analysis is a process consisting of three components: risk assessment, risk management and risk communication. Today, the new approach is considered as an approach enabling food safety issues to be diagnosed more accurately and define strategies required to decrease these issues more effectively [23–25]. The principles of risk-based food safety are defined with a four-step framework. The first step includes a series of initial risk managements such as defining the food safety issues, developing a risk profile, setting risk management goals, deciding on the need for a risk assessment, forming a risk assessment policy, creating a risk assessment and/or risk ranking commission and analysis of the results following the assessment. In the second step, different risk management options are defined and the options are chosen after the assessment. The third step includes the implementation of risk management precautions. Lastly, in the last step, observations are carried out in appropriate areas within the food chain and this step is utilized in reviewing the effectiveness of the risk management precautions. This step usually includes public health monitoring in order to collect data on changes. In summary, this approach aims at improving the food safety in high-risk food/hazard combinations, decrease the burden of foodborne diseases and increase the consumer safety [25].

Billions of people in the world are under unsafe food risk. Each year, hundreds of thousands of people become sick or lose their lives due to consumption of unhygienic, high-risk foods. This is why safe food saves lives. In addition to improving the health of individuals and the public, safe food also boosts the economic growth in the regions where it is improved. Food safety covers four main areas, as shown in **Table 2** microbiological safety, chemical safety, personal hygiene and environmental hygiene [26].

Microbiological safety: the potential sources of foodborne diseases are bacterial agents. Diseases can range from mild gastroenteritis to neurological, hepatic or renal syndromes. Foodborne bacterial agents are primary cause of severe and fatal foodborne diseases. More than 90% of food poisoning diseases are caused by <i>Staphylococcus</i> , <i>Salmonella</i> , <i>Clostridium</i> , <i>Campylobacter</i> , <i>Listeria</i> , <i>Vibrio</i> , <i>Bacillus</i> and <i>E. coli</i> types.
Chemical safety: foods may contain some non-food chemical additives such as coloring agents or preservatives and contaminants such as pesticide residues. Heavy metals such as lead, cadmium, mercury and copper can be found in some food products possibly because of kitchen appliances or inadequate food hygiene.
Personal hygiene: inadequate personal hygiene in food processors or preparers can pose a great risk to public health. Simple activities such as hand washing and adequate washing facilities can prevent many foodborne diseases.
Environmental hygiene: inadequate or wrong recycling and lack of equipment for disposing of wastes lead to accumulation of spoiled and contaminated food. This situation than leads to the increase in the insect and bug populations contributing more to the risk of contamination and spoiling. For this reason, the hygienic conditions of the areas where food is processed and prepared are very important.

Table 2.
Four main areas of food safety [26].

4. Storage conditions and hygiene in foods

Controlling the entry of contaminants into the food chain can be difficult. In addition to poor hygiene, unfavorable transfer and storage conditions for foods or contaminated raw material usage also play a part in contamination. Low quality or contaminated foods may cause shipments to be canceled on an international level. This poses an obstacle for the trade between countries [27]. Food safety objectives are based on preventive actions such as safe raw material usage, good production practices and procedures with critical control points for hazard analysis. It is possible for the success of these preventive actions to reflect on the incidence of foodborne diseases. WHO and Center for Control of Foodborne Infections and Intoxications in Europe stated that one of the most important factors contributing to foodborne outbreaks were markers required for improving general hygiene and most of these were under the control of producers/consumers and listed these markers as following:

- Poor general hygiene
- Consuming raw products
- Using contaminated materials
- Contamination through infected people
- Cross-contamination
- Using contaminated tools
- Mistakes in processing
- Too early preparation
- Inadequate heating
- Inadequate warm-keeping
- Inadequate cooling
- Too long storage time
- Contamination during the last preparation phase
- Inadequate heating before reusing [28].

Attention should be paid to purchasing, preservation, preparation, cooking and serving processes for ensuring food hygiene and safety. While purchasing foodstuffs, attention should be paid to the shipment conditions, packaging and keeping the cold chain in potentially high-risk foods such as fish, meat, chicken and milk. Storage rules should be followed during storing. First in first out (FIFO) rule should be followed in storages. Temperature in storage units should be checked regularly and cooked meals should be left to cool down in room temperature before being stored in fridges. Shelves should be made of rustproof material and foods should be kept at least 15 cm away from the floor and walls.

Food	Preservation time (day)
Big piece of meat	3-5
Chicken	2-3
Minced meat	1-2
Sausage	2-3
Cooked meat	2-3
Raw fish	1-2
Shellfish	1
Cooked fish	2-3
Milk and cream	3-4
Eggs	14
Fruits	1-14
Vegetables	2-7

Table 3.
Preservation time for some foods [22].

There should be different sections for each food group (meat group, dairy group, fruit and vegetable group) so that cross-contamination is prevented. There is a risk of microorganism contamination from personnel, tools, environment or foods (cross-contamination) during the preparation phase. Color code system could be implemented in cutting areas to be able to prevent this from happening. Potentially high-risk foods should be processed without waiting. Cooked meals should be served in maximum 2 hours. Frozen foods should be thawed in 4–7°C. Internal temperature of poultry should be at least 75°C while cooking. Temperature of foods such as meat, fish and eggs should be increased to at least 63°C and they should be processed at this temperature for at least 2 minutes. Internal temperature of hot meals should be kept at 65°C in bain-marie with a closed lid. While serving food, clean containers should be used to transfer or hold the food. Cold foods should be kept under 4.5°C in a closed container. Preservation time is as important as preserving conditions when it comes to development, growing and spreading of microorganisms. Preservation times for some foods are listed in Table 3 [22, 29].

5. Different pathotypes of *E. coli* and outbreaks

If we take a general look at the incidence and epidemiology of disease-causing *E. coli* pathotypes, we see many cases and outbreaks. For example; annual incidences of 31 primary pathogens were estimated in a study conducted in the USA in 2011. It is estimated that these 31 pathogens caused 6.6–12.7 million diseases; 39,500–75,700 hospitalizations and around 700–2300 mortalities. In another study conducted in the USA between 2003 and 2012, it was reported that foodborne outbreaks caused 4928 diseases, 1272 hospitalizations, 299 cases of HUS diagnosed by a physician and 33 deaths. The primary contamination sources were listed as 55% foodborne, 10% animal contact, 10% human-to-human transmission, 4% waterborne and 11% unknown reasons [28, 30]. In another study conducted in Argentina, O157:H7 STEC was detected 25.5% and non-O157 STEC was detected in 52.2% of the raw meats analyzed in terms of STEC. Argentina is one of the countries with the highest HUS incidence rates [31].

In meat products, non-O157 STEC prevalence varies between 2.4 and 30.0% for minced meat, 17.0 and 49.2% for sausage and 8.6 and 49.6 in meat put up for sale. When STEC contamination reports verifying that the STEC O157 prevalence had ranged between 0.2 and 27.8% for the last 30 years were assessed in terms of STEC O157 and non-O157 presence in bovine meat, non-O157 STEC rates were observed to be ranging between 2.1 and 70.1% [32]. Besides, EHEC serotypes were reported to stay alive for 9 months in -80°C and that they were not affected by the storage conditions of pieces of meat frozen in -20°C . *E. coli* is reported to be directly associated with consumption of undercooked meat. It is known that, especially meat and milk are very suitable environments for verotoxigenic *E. coli* and this microorganism produces significant amounts of verotoxins in contaminated meat kept in 37°C [33]. In studies, it was reported that the foods that are generally responsible for foodborne outbreaks were: meat and meat products, fish and seafood, chicken products, liver, ice cream, raw milk, rice meals, pasta and pasta salad, peanut, flour, cold sandwiches, fruit juices and raw fruits and vegetables [23]. In another study, it was stated that undercooked or uncooked hamburger, non-pasteurized fruit juices, raw vegetables contaminated with cow manure and infected cows are important sources of *E. coli*. For example; there were at least four deaths and over 500 laboratory approved infections were observed in an *E. coli* outbreak in 1993, related to hamburgers bought from a fast-food chain [6, 23]. The outbreak in the west of USA between 1992 and 1993 caused by *E. coli* O157:H7 that affected over 700 people and the outbreak in Japan in 1996 that affected over 8000 people and killed two people can be listed among the outbreaks caused by *E. coli* O157:H7. Foodborne outbreaks caused by *E. coli* O157:H7, O111:NM (non-motile) and STEC serotypes were reported in many countries such as Australia, Canada, Japan, USA, many European countries and North Africa [34].

In a study conducted on children's nursery in Japan between 2010 and 2013, it was detected that 68 of 1035 outbreaks were of EHEC origin. It is known that 30 of the 68 outbreaks (46%) were foodborne [35]. It is also known that there were two EIEC outbreaks reported in England in June of 2014. These cases are rare in England. However, it is emphasized that EIEC has a capacity to cause large and potentially serious gastrointestinal outbreaks in Europe and that it should be considered as a potential pathogen in foodborne outbreaks [36]. In 2011 (between May 1st and July 4th) 2971 STEC related gastroenteritis cases including 18 deaths and 845 HUS cases including 36 deaths were reported along with laboratory approval, among 3816 cases reported to the public health officials in Germany. Moreover, the number of HUS cases during outbreaks was reported to be approximately 70 times the figures that corresponds to the same period of previous years [37]. In another report from Germany, a case-control study was conducted with 26 patients with HUS and 81 control cases. The incidence of the disease was associated with kale consumption in the univariate analysis and with kale and cucumber consumption in the multivariate analysis. Twenty-five percent of the cases reported eating kale and 88% reported eating a salad [38]. In another case in Scotland in 1994, 71 cases were reported including 1 death and 11 HUS cases due to non-pasteurization of milk. In an *E. coli* O111 outbreak in Australia, 200 cases were reported including 23 HUS cases and 1 death due to a kind of sausage made from minced meat. The failure in chlorination of Municipality waters also caused outbreaks. In an outbreak in 2000 among campers in Aberdeenshire, 20 cases were reported due to the environmental exposure to the camp area contaminated by sheep manure. Among this group of campers, the number of people that the number of cases who had not washed their hands before meals was almost 9 times bigger than the number of people who became ill. It is a

well-known fact that all *E. coli* outbreaks cause high costs for countries in addition to the severity of the infection and the damage it leaves on people [39].

6. Conclusion

Along the food chain, controllability and traceability are of great importance for ensuring the consumer safety and for foods to be protected from biological, physical and chemical hazards starting from the field to the moment of consumption. Consumers constitute the last ring of the food safety. The purchasing power and consciousness of consumers help ensure food safety and are the most important factors for protection and prevention against risks. Prevention of *E. coli* infections require not only developing new vaccines but also providing uncontaminated water and food. Food production companies should pay close attention to the cleanliness of their application areas and the disinfection of the running water. People who work in food facilities and services, should be given frequent trainings on hygiene so as to prevent *E. coli* contaminations. During travels from developed countries to developing countries, unsafe foods and foods that are sold out in the open should be avoided; packaged and labeled drinking water and beverages should be consumed [2, 40]. Cooking food at the right temperatures can ensure inactivation of *E. coli* as the factor is sensitive to high temperatures. While the meat is cut into pieces, the microorganisms on the surface of the meat reach the inner sections and can stay alive if a sufficient heat treatment is not applied, turning it into a risk factor for public health. Similarly, there are some potential risks in raw milk. It poses a risk if not pasteurized. *E. coli* can be inactivated with pasteurization [15, 41, 42].

Biological protection precautions are also very important. It is claimed that $8.0 \log_{10}$ cfu/g lactic acid bacteria causes a $1.6 \log_{10}$ cfu/g decrease in *E. coli* O157:H7; EHEC O157 multiplies by growing in the damaged Fuji apple, yet *Candida oleophila* may be effective in controlling this pathogen in these damaged apples. Decreasing this risk of contamination caused by farms, slaughterhouses, food producers and consumers is very important for protection from the pathogen *E. coli* O157:H7 strain, which can also be transmitted through food and water. Under the Food and Drug Administration (FDA), Meat Inspection Act and other regulations the food industry is responsible for producing safe foods that meet national standards, identify critical control points from production to consumption, and have good production practices. 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 for raw material production, procurement and packaging, distribution and consumption [2, 17, 40, 43].

Under the HACCP, the term hazard refers to any substance or condition that has the potential to cause adverse health effects and that is unacceptable. These hazards can be caused by the biological, chemical or physical contamination in the raw material, semi-processed or finished food product. Hazard analysis is defined as the assessment of the severity of the hazard and the likelihood of it happening. HACCP is a system managed based on seven principles to identify, assess and control possible hazards for food [17, 44];

1. Conduct hazard analysis
2. Identify critical control points (CCP)
3. Establish critical limits

4. Establish monitoring procedures
5. Establish corrective actions
6. Establish verification procedures
7. Establish documentation and record procedures [17].

These principles are accepted by state institutions, trade associations and the food industry. Today, food safety systems based on HACCP principles are successfully implemented in food processing facilities, retail food stores and global food service operations. Following HACCP rules in production facilities is vital. In a slaughterhouse in Mexico it was emphasized that HACCP should be applied in addition to antimicrobial treatment to reduce the presence of potential pathogens such as *E. coli* O157: H7 and non-O157 STEC in cattle carcasses. In the USA, it was stated that the most stringent measure for the prevention and control of EHEC is to determine the critical control points that lead to contamination of meat. Good manufacturing practices (GMP) and standard sanitation operating procedures (SSOPs) are accepted as the first steps in developing the HACCP system in the food industry. Successful implementation of GMPs and SSOPs is of great importance for the HACCP, because these systems are the building blocks of food safety during the processing phase [45]. These guidelines act as a guarantee for production, test, quality and assurance to help reduce the risk of foodborne diseases and ensure production and distribution of safe food for human consumption. Many countries follow GMP procedures and established their own GMP rules in accordance with their own legislations. The purpose of GMP is also help reduce the uncontrollable risks such as contamination and cross-contamination by testing the product. The main requirements for GMP are listed below [43, 45, 46];

- Comprehensible written instructions and procedures
- Trained employees
- Records of actions, mistakes and reviews
- Records of production and distribution
- Proper storage and distribution
- Complaint and recall systems [46].

In conclusion; it should not be forgotten that as a foodborne pathogen *E. coli* can spread in food, even in small numbers, and has the potential to cause infections, food poisoning and even death. Preventive measures include protecting the food from direct or indirect contamination, applying personal hygiene practices, preserving the processed food in appropriate places and temperatures, checking proper packaging and proper storage, cooking in proper temperatures, allowing proper cooling and keeping the cooked food away from raw food. There are many simple measures for consumers to take in order to prevent bacterial growth and ensure food safety. Consumers can develop their own safety methods at home by following the abovementioned measures. It is very important for food producers to comply with the safety method principles such as HACCP and GMP in terms of public health so as to prevent many diseases and outbreaks.

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