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Food threats in organic produce through biological contaminants

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Abstract
Food borne pathogens acts a main risk factor in terms of public health in the developed and developing countries caused by their adequateness all around the world. The most important foodborne pathogens are *Escherichia coli* and other coliform bacteria. Some other most important cause of contamination for these microorganisms are described as: places with unfavourable hygiene, contaminated waste water, meat, cereal, fruits and vegetable products. Overall coliform bacteria and *E.coli* count is known as the sign of unfavourable sterile conditions and fecal contamination in foods. Foodborne diseases are still regarded as a major global issue. A common approach by all the countries and other international organizations is a precondition for identifying and controlling foodborne problems that cause a threat to human health and international market. By observing their problematic biology, epidemiology and analyses it is observed that most of the foodborne diseases are curable. It is important for public health that purchasers and food producers should act in accordance with the concept of internationally accepted safety methods.

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

Introduction
Food safety is defined as the handling, preparing and storing the food in such a way to reduce the risk of becoming sick. The main aim of food safety is to prevent the food from getting contaminated in order to avoid food poisoning.
There are several biological contaminants which play a major role in food safety and quality such as manure, *E.coli*, Mycotoxins, contamination from natural fertilizers. Foodborne pathogens are regarded as an important factor for public health in the developed and developing countries die to their adequateness all around the world. The most important foodborne pathogenic bacteria is *Escherichia coli*.
E.coli is defined as the bacteria that live in the intestines of animals and humans. *Escherichia coli* is considered as an important pathogenic bacteria and is transmitted through fecal route and cause diarrhea genic effect. It is regarded as an indicator bacteria in food safety and hygiene. The presence of *E.coli* in fruits and vegetables plays an important role in inadequate hygiene.
E.coli are considered as the harmless to human (Croxen and Finlay 2010). It can cause diarrhea. Every year, there are nearly 1.7 billion cases of diarrheal disease. *E.coli* are able to express virulence traits through which they cause a variety of diarrheal disease syndromes. As it is estimated that on any single day 200 million people are affected by this diarrheal illness. Production of Shiga toxin 2 and the adhesion intimin are two important factors for *E.coli* O157. According to the report, it was found that the presence of *E.coli* in organic produce samples was 9.7% as compared to the conventional produce was 1.6%. However, it's prevalence in certified organic farms was 4.3% that is not higher than the level in conventional produce whereas it's prevalence in uncertified organic farms produce was 11.4% which is higher than in the certified organic produce.
According to the previous reports, it was found that *E.coli* was 19 times more widespread in produce from organic farms that use manure/ compost less than a year old as fertilizer when compared with the organic farms that use older materials. The other source of contamination is through irrigation includes Wells, rivers and lakes. It had been reported by Islam *et al*. that pathogens such as *S. Typhimurium* and *E.coli* O157:H7 can live in soil for longer period (>150days) and in vegetables (>60 days). Food safety means to ensure all the necessary hygienic conditions and taking all the safety precautions for a healthy and safe food production starting from raw material to consumption of food.

Other source of contamination is equipments used in post harvest handling and poor hygiene can increase the effect of microbial contamination. In order to prevent microbial contamination proper storage conditions should be available including proper temperature, air circulation and relative humidity. To prevent these illness hygiene, food preparations, availability of clean water and controlling contamination of the environment are necessary.

So, this section will mainly focus on the pathogenic characteristics of the food that are contaminated with *E.coli*, food contamination cases, current food safety approaches and the various methods of protection.

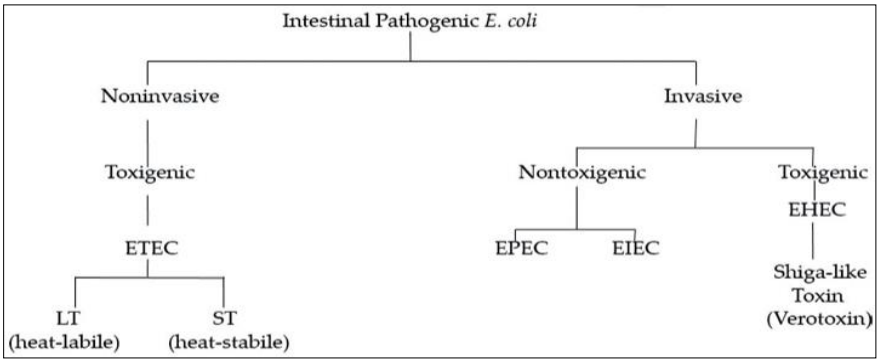
Escherichia coli and Food poisoning

Escherichia coli is the 30 members of the bacterial family of Enterobacteriaceae. *Escherichia coli* is a coliform bacterium and it is one of the 6 types of Escherichia species (*E. adecaroxylate*, *E. blattae*, *E. fergusonii*, *E. hermannii* and *E. vulneris*). *E.coli* is a gram negative, anaerobic, rod shaped, mesophilic bacterium that grows in 7-45°C. *E.coli* is found in lower intestine of the warm blooded organisms. They are found in the intestines of both healthy people and animals. Coliform bacteria consists of Citrobacter, Enterobacter, Klebsiella and Escherichia. The presence of coliform group in food can be determined through fecal contamination and poor sterile conditions. For example, the presence of coliform bacteria in raw milk is an indicator of poor hygiene in milking. It's presence in raw fruits and vegetables is not very important as Enterobacter, Klebsiella and Citrobacter, they are naturally present in the microbiota of plants. The presence of *E.coli* in fruits and vegetables is important for inadequate hygiene. *E.coli* is an indicator of fecal contamination in foods and drinking water. Due to this feature, it is regarded as an indicator bacterium in food safety and hygiene (Erkmen O 2013) [3].

Some of its pathogenic species both cause intoxication by creating toxins and cause gastroenteritis, pathologic kidney and brain damage by causing toxins of a type of infection through cellular increase. Some enterotoxin that produce *E.coli* strains are divided into heat-stable and heat- labile. Heat-stable toxins are known as stable toxin (ST) and heat-labile toxins are known as labile toxin (LT).These toxins can be found together or individually. These pathogenic strains also causes serious diseases such as pneumonia, diarrhea, mastitis. *E.coli* also causes meningitis especially in neonatal period. It has a high rate of illness and death worldwide. The mortality rates in neonatal meningitis are reported to vary between 15–40% and 50% of survivors are reported to continue their life with neurological damage (Donnenberg MS 2017) [4].

The pathogenic intestinal coli is classified as six subgroups such as enterotoxigenic *E.coli* (ETEC), Enteropathogenic E. *E.coli* (EPEC), Enteroaggregative *E.coli* (EAEC), E. DAEC), enteroinvasive *E.coli* (EIEC) and enterohemorrhagic *E.coli* (EHEC). EHEC is also known as Shiga toxin (stx) which produces *E.coli* (STEC) and E-producing verotoxin. *Coli* (VTEC).

Mechanism of intestinal pathogenic *E.coli* strains (Gerba CP. 2014) [6].



Enterotoxigenicity *E. coli* (ETEC)

People who are living in developing countries are often reported to have this present in the feces and show that they have developed immunity against this microorganisms. As the cause of death of children under 5, the microorganism most commonly seen in childhood diarrhea is ETEC and is responsible for 30-60% traveler's trauma. The infection is characterized by wet diarrhea and, depending on the individual, its movement may range from a normal course to a cholera-like exposure with the addition of symptoms such as vomiting and high fever (Zhang W, Sack DA 2015) [7]. The most common cause of mortality among young children is diarrhea and is found in people living in Asia and Africa with inadequate hygiene. It have been reported that every year an estimate of 600,000 children who are under the age of 5 lose their lives. The main cause of diarrhea occurrence is the consumption of food or water contaminated with bacteria or parasitic pathogens. With the production of fimbrial or non-fimbrial adhesins, ETEC strains cause water hyper secretion by producing enterotoxins that break fluid homeostasis and electrolyte in epithelial cells of the small intestine that lead to watery diarrhea. Without rehydration, moderate or severe diarrhea can lead to dehydration and serious death (Zhang W, Sack DA 2015) [7].

Entomopathogenic *E. coli* (EPEC)

It is one of the oldest known *E.coli* strain that causes diarrhea. It causes a serious risk to human health and causes infant mortality in the developing countries. Humans and pigs are infected by this microorganism. It is transmitted from person to person and it is known to spread through contaminated water and food (Gerba CP 2014) [6].

It is classified as typical EPEC (tEPEC) and atypical strains (aEPEC). These EPEC strains lack Shiga toxin producing genes. The main pathogens associated with chronic diarrhea are EPEC, ETEC and EAEC. aEPEC was the most common pathogen among children with chronic diarrhea. These detection shows that atypical EPEC have tendency to be more chronic than other diarrheagenic *E. coli* (Ochoa TJ, Contreras CA 2011) [8].

Enter aggregative *E. coli* (EAEC)

It is a foodborne pathogen that is observed in critical and persistent diarrhea cases in children and patients with repress immune system. The first stage is the strong adherence to the intestinal mucosa, the second stage leads to the development of enterotoxins and cytotoxins and the third stage is known for its ability to induce mucosal inflammation.

It is commonly found in Mexican foods, which includes desserts and salsa sauces, and visitors of that country are known to be more sensitive to EAEC diseases during their stay than ETEC, which is more affected by them. The reason for this is EAEC's ability to suppress the immune system and cause chronic infections. It is more resistant to antibiotics. Persistent infection and chronic disturbance in Intestinal functions can lead to malnutrition that is caused due to deficiency of micronutrients that induces infection. This entire cycle increase the load of acute diarrhea (Okhuysen PC, DuPont HL 2010) [10].

Diffusely-Adherent *E.coli* (DAEC)

HeLa cell cultures are known as DAEC due to their dispersed adherence features. They can cause chronic diarrhea in children between the age of 1 and 5. The infection can be indicated by mild diarrhea void of fecal leukocytes. In France, DAEC strains were found to be more prevalent in cases of diarrhea seen in patients hospitalized without enteropathogen. This situation suggests that DAEC strains may be a significant diarrhea in developed countries. Recent research shows that some DAEC species contain virulence factors in the uropathogenic *E. coli* (UPEC) strains.

Enteroinvasive *E.coli* (EIEC)

EIEC strains that cause inflammatory damage to the intestinal mucosa and submucosa are very similar to those produced by Shigella. These microorganisms have the same ability to disperse and reproduce within epithelial cells. However, EIEC-related watery diarrhea is more commonly diagnosed than dysentery caused by Shigella. Symptoms include weakness, abdominal cramping, fever, watery stool and difficulty in urinating. The incubation period is examined as 10-18 hours. EIEC is transmitted through contaminated foods. Enteroinvasive Strains can be treated by antimicrobials that are effective against Shigella isolates.

Enterohemorrhagic *E. coli* (EHEC)

EHEC are also called as Shiga toxin producing *E. coli* and verotoxin producing *E. coli*. Based on the severity of the disease, EHEC is considered to be the most severe form of *E.coli* among foodborne pathogens. EHEC has a broad spectrum consists of watery or bloody diarrhea, which is considered as an important factor in acute renal failure in children. The largest outbreak of EHEC O104: H4 in 2011 was in Germany with 855 HUS cases of 3842 people and 53 deaths. This incident, which raised concerns around the world, demonstrates the importance of EHEC in terms of human health. sHUS can develop 1 week after the onset of diarrhea, which can lead to death especially in children and the elderly. HUS is characterized by renal acute failure, hemolytic anemia and thrombocytopenia. Coma, stroke, colon perforation, pancreatitis and high blood pressure have been included in other HUS complications. It is estimated that it leads to the early development of chronic kidney failure in 15% of cases. Dialysis is required in HUS patients and the mortality rate is 35%. In addition, it is more commonly seen in women (70%) and during pregnancy (13%).Good treatment for this infection is still lacking, however; other new treatment strategies such as the use of antibodies against anti-vero toxin (anti-Shigatoxin) have been suggested. TTP, on the other hand, is clinically similar to HUS and fever, abdominal pain, intestinal bleeding and central nervous system disorders are listed among the possible complications. Often, it builds up blood clots in the brain and causes death (Ertaş N, Yıldırım Y, Karadal F, Al S.2013).

Table 1: Summary of incidence and epidemiology of *E.coli* serotype

Pathogen	Area of infection	Disease	Target population	Transmission route
EPEC	Small intestine	Diarrhea	Children	Transmit through water and infant formula
EHEC	Large intestine	Hemolytic uremic syndrome (HUS)	All ages	Transmit through food(beef produce)
EIEC	Large intestine	Cause Dysentery (bloody diarrhea)	Children	Transmit through water
EAEC	Intestine	Watery diarrhea	Children and adults	Transmit through food and water
ETEC	Small intestine	Diarrhea, chronic diarrhea	Children and travellers	Transmit through food and water

***Source*:** Gerba CP. environmentally transmitted pathogens. In: Environmental Microbiology. 3rd ed. Elsevier Inc; 2014.pp.509-550

Food safety and High-risk foods: Food safety means protecting the products from biological, physical and chemical hazards starting from field to processing, storing, distribution, preparing and cooking. In several countries all over the world, people begin to have new conscious perspective on food safety and environment. Consumers contribute to prefer food that is extra healthy, environment friendly, less processed, natural and produced safely. Pro-active approach that is based on the risk analysis and it is the most suitable and effective method for controlling foodborne disease and pathogens. It also involves the function of proper control systems in the production chain (Seydi A, 2017) [14]. Foodborne diseases are a worldwide subject. A familiar approach taken by all the countries around the world and other international organization is essential for observation and control of foodborne pathogen problem threatening human health and international trade. Although their complicated biology and epidemiology, most of the foodborne pathogens are preventable. Food industry, health institutions and consumers must be dedicated to prevent foods from becoming contaminated at restaurants, bars, farms and homes. In the outbreak of foodborne pathogens, continuous monitoring is essential to identify food allergies, regions and related pathogens. Genotype and subtype information obtained from contaminated species is required to trace the source of contamination, to estimate and to compare species (Bintsis T 2017) [31].

Classic food safety management systems that were once adopted for safe production and utilization of food appear to be ineffective and researchers/organizations proposed a "risk-based food safety" approach. The risk-based approach to food safety is very different from the traditional risk-based approach. In this regard, the food safety management plan aims at calculating the risks to human health and to define,

select and implement strategies to manage and reduce these risks. According to Codex Alimentarius, risk analysis is a three-part process: risk assessment, risk management and risk communication. Today, the new approach is seen as a way to allow food safety issues to be more accurately identified and to define strategies needed to reduce these problems more effectively (Barlow SM, Boobies AR, Bridges J, Cockburn A, Dekant W, Hepburn P, *et al*. 2015) [19].

The concept of risk based food safety are defined into four-step structure. The first step consists of a sequence of initial risk management like food safety issues, setting risk management goals, forming a risk assessment policy, creating a risk assessment. The second step consists of different risk management options and the options are selected after the assessment. The third step consists of performance of risk management precautions. At last step, Observations are finalized in appropriate areas within the food chain. This step is adapted in reviewing the effectiveness of risk management precautions. This aims at improving the safety of food at high risk food, reduce the burden of foodborne pathogens and helps to increase the consumer safety (Barlow SM, Boobies AR, Bridges J, Cockburn A, Dekant W, Hepburn P, *et al*. 2015) [19].

Every year, millions of people become sick and lost their life due to consumption of unhealthy and high-risk foods. Food safety includes four major areas, microbiological safety, personal hygiene, chemical safety and environmental hygiene (Fung F, Wang H, Menon S, 2018) [20].

Four main areas of Food Safety

Environmental hygiene: Inadequate or incorrect recycling and lack of waste disposal equipment leads to the accumulation of spoiled and contaminated food. This condition instead leads to an increase in the number of insects and bug population that contribute to the risk of infection and contamination. For this reason, hygienic conditions in areas where food is processed and prepared.

Microbiological safety: Possible sources of foodborne pathogens are bacterial infections. Diseases can range from central gastroenteritis to neurological, hepatic or renal syndromes. Bacteria in food are a major cause of serious foodborne illness. More than 90% of foodborne illnesses are caused by Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus and *E. coli*. Species.

Chemical safety: Food consists of non – food chemical additives like Preservatives, coloring agents and pesticides residue contamination. Heavy metals such as lead, cadmium, mercury and copper can be found in some food products either because of kitchen utensils or food contamination.

Personal hygiene: Inadequate personal hygiene in food processors or preparers can be extremely dangerous to public health. Simple tasks such as hand washing and adequate laundry can prevent many foodborne illnesses.

Storage conditions and hygiene in foods

It can be difficult to stop the entry of contaminants into the food chain. In addition to poor hygiene, poor conditions of transmission and storage of food or the use of raw materials also contribute to contamination. Low quality or contaminated food can cause shipments to be canceled internationally. This puts a barrier to international trade (Korada SK, Yarla NS, Putta S, Hanumakonda AS, Lakkappa DB, Bishayee A, *et al*. 2018) [29].

The objectives of food safety are based on preventative actions such as good production practices, safe raw material usage and procedures with crucial control points for hazard analysis. The WHO and the Center for Control of Foodborne Infections and Intoxications in Europe said that one of the most important factors contributing to the food crisis was the markers needed to improve general hygiene and most of them were under the control of manufacturers/consumers and listed the following symptoms:

- Poor hygiene condition
- Eating raw materials
- Using contaminated products
- Contamination occur through infected person
- Cross contamination between people
- Using contaminated equipments
- Mistakes during processing
- Improper heating method
- Inappropriate cooling
- Storage time is too long
- Contamination occur during last preparation stage
- Improper heating before use (Notermans SH. In: Batt CA, Tortorello M, 2014) [30].

Proper care should be taken before purchasing, preparation, preservation, cooking and serving processes for maintaining food safety and hygiene.

Table 2: Time of preservation for some food items

Food items	Time duration for preservation
Chicken	2-3 days
Sauces	2-3 days
Raw fish	1-2 days
Cooked fish	3 days
Milk and cream	3-4 days
Egg	14 days
Fruits	1-14 days
Vegetables	2-7 days
Shellfish	1 day

***Source*:** Seydi A.2017 [14]

In order to prevent cross contamination there should be different groups for each food (meat group, fruit and vegetable group and dairy group). During the preparation stage, there is a chance of getting microorganisms contamination from personnel, tools, environment. The color code system can be used in cutting areas so you can prevent this from happening. Foods that are potentially dangerous should be considered without waiting. Frozen foods should be stored at 4-7 °C. The internal temperature of the chickens should be at least 75 °C while cooking. The temperature of foods such as meat, fish and eggs should be raised to at least 63 °C and should be considered at this temperature for at least 2 minutes. The internal temperature of hot food should be kept at 65 °C in a bain-marie with a closed lid. When serving food, clean utensils should be used to transfer or handle food. Cold foods should be stored below 4.5 °C in a sealed container. Preservation time is just as important as preserving conditions when it comes to developing, growing and distributing microorganisms.

Different types of *E. coli* patho types and their outbreaks

In general, we see the incidence and epidemiology of *E.coli* causing diseases, we see many conditions and outbreaks. For

example; annual cases of 31 viruses were estimated in a 2011 study in the USA. It is estimated that these 31 diseases caused 6.6-12.7 million diseases; 39,500-75,700 hospitalization and death of approximately 700-2300. In a study conducted in the USA between 2003 and 2012, it was reported that foodborne illness caused 4928 infections, 1272 hospitalizations, 299 HUS-physician cases and 33 deaths. The main sources of contamination were recorded as 55% of food, 10% of animal contact, 10% of human-to-human transmission, 4% of water and 11% of unknown causes (Heiman KE, Mody RK, Johnson SD, Griffin PM, Gould LH, 2015) [16].

In another study conducted in Argentina, O157: H7 STEC found 25.5% and non-O157 STEC found 52.2% of raw meat analyzed according to STEC. Argentina is one of the highest HUS incidence rates countries (Castro VS, Carvalho RCT, Conte Junior CA, Figuiredo EES, 2017) [17].

In the study, it was reported that the most common foods that are responsible for foodborne outbreak were: Meat and meat products, fish and seafood, poultry products, liver, ice cream, raw milk, rice foods, pasta and pasta salads, nuts, flour, cold sandwiches, juices fruits and vegetables and raw vegetables (Bintis T 2017) [31].

In another study, it was reported that raw or undercooked hamburgers, raw fruit juices, raw vegetables contaminated with cow dung and infected cattle were important sources of *E.coli*. For example; at least four people died and more than 500 laboratory-sanctioned diseases were detected in the outbreak of *E. coli* in 1993, related to hamburgers bought at a fast food restaurant (Gerba CP 2014) [6].

In a study conducted in a preschool in Japan in the year between 2010 and 2013, it was found that 68 of the 1035 infections were from EHEC. It is known that 30 out of 68 outbreaks (46%) were caused by food borne pathogens (Kanayama A, Yahata Y, Arima Y, Takahashi T, Saitoh T, Kanou K, *et al*. 2015) [32].

It is also known that in June 2014, there were two EIEC outbreaks reported in England. However, it is emphasized that EIEC has the potential to cause large and potentially fatal intestinal infections in Europe and should be considered a potential virus for food allergies (Newitt S, MacGregor V, Robbins V, Bayliss L, Anne Chattaway M, Dallman T, *et al*. 2016) [22]. In the year 2011 (between May 1st and July 4th) 2971 STEC cases related to gastroenteritis including 18 deaths and 845 HUS cases including 36 reported deaths and laboratory accreditation, among 3816 cases reported to public health officials in Germany. Moreover, the number of HUS cases during the outbreak was reported to be 70 times the number corresponding to the same period of previous years (Frank C, Werber D, Cramer JP, Askar M, Faber M, Heiden M, *et al*. 2011) [24].

In another study it was reported from Germany, a case-control study was conducted with 26 patients with HUS and 81 control cases. The incidence of this disease was associated with the use of kale in informal analysis as well as the use of kale and cucumber in multivariate analysis. Twenty-five percent of cases reported that they ate kale and 88% reported eating a salad (Buchholz U, Bernard H, Werber D, Böhmer MM, Remschmidt C, 2011) [25].

It is known that all *E.coli* pathogens cause high costs of Contamination for countries in addition to the severity caused by infection and the damages that it leaves on people (Pennington H 2010) [36].

Conclusion

On the food side, controllability and traceability are crucial in ensuring consumer safety and that food is protected from biological, physical and chemical hazards from the field to the time of consumption. Consumers are building a final food safety ring. Purchasing power and consumer awareness helps to ensure food safety and are the most important factors for food safety and security risks. Prevention of *E.coli* diseases requires not only the development of new vaccines but also the provision of water and non-contaminated food. Food companies should pay close attention to the hygiene of their application areas and to the disinfection of running water. People who work in restaurants and facilities, should be regularly trained in hygiene to avoid *E.coli* contamination.

Under the Food and Drug Administration (FDA), Meat Inspection Act and other regulations the food industry is responsible for producing safe food that meets national standards, identifies control areas from production to consumption, and has good production processes. Hazard Analysis and Critical Control Points (HACCP) is a management plan in which food security is addressed through the analysis and control of biological, chemical and physical hazards of raw material production, procurement and packaging, distribution and use (Hurd HS, Malladi S.2012, Cálix-Lara TF, Rajendran M, Talcott ST, Smith SB, Miller RK, Castillo A, *et al*., 2014) [27, 23].

HACCP is a managed program based on seven principles for identifying, evaluating and controlling potential food hazards (Ibrahim OO, 2015) [33].

1. Performing hazard analysis
1. Determine critical control points (CCP)
2. Establishing critical limits.
3. Determining monitoring procedures
4. Establishing corrective measures
5. Determining verification procedures
6. Establishing documentation and recording procedures. (Ibrahim OO, 2015) [33].

These policies were adopted by government institutions, trade unions and the food industry. Today, HACCP-based food safety systems are successfully used in food processing facilities and worldwide food service operations.

In conclusion; it should not be forgotten that the pathogenic *E.coli* found in food it can spread to food, even in small numbers, and has the potential to cause disease, food poisoning and even death. Preventive measures include protecting food from direct or indirect contamination, using personal hygiene practices, maintaining processed foods and temperatures, checking proper pack packaging and storage, cooking in the right temperature, allowing proper cooling and keeping cooked food away from raw. Food. There are many simple steps consumers can take to prevent the growth of bacteria and to ensure food safety. Consumers can build their own home safety systems by following the steps outlined above. It is very important that food producers adhere to safety standards such as HACCP and GMP in public health to prevent many diseases and outbreaks.

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