Magna Scientia Advanced Research and Reviews

eISSN: 2582-9394

Cross Ref DOI: 10.30574/msarr

Journal homepage: https://magnascientiapub.com/journals/msarr/

(REVIEW ARTICLE)

Pathogenic *Escherichia coli* (*E. coli*) food borne outbreak: Detection methods and controlling measures

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Magna Scientia Advanced Research and Reviews, 2024, 10(01), 052–085

Publication history: Received on 1 November 2023; Revised on 20 December 2023; Accepted on 12 January 2024

Article DOI: https://doi.org/10.30574/msarr.2024.10.1.0003

Abstract

Food-borne illnesses and diseases are major threats to human health. Common food borne pathogens include bacteria such as *Escherichia coli* Serogroup (O157), *Bacillus cereus*, *Clostridium botulinum*, *Campylobacter* sp., *C. perfringens*, some *Listeria monocytogenes*, *Salmonella* spp., Shigella spp., *Staphylococcus aureus*, and Vibrio spp. etc. Pathogenic *E. coli* infection usually causes severe diarrhea. *Escherichia coli* is the most common cause of acute urinary tract infections as well as urinary tract sepsis. Diarrheagenic *E. coli* pathotypes can be passed in the feces of humans and other animals. Safeguarding public health during mass gatherings is a big challenge. Despite the recent advances in food preservation techniques and food safety, significant disease outbreaks linked to food borne pathogens such as bacteria, fungi, and viruses still occur worldwide indicating that these pathogens still constitute significant risks to public health. However, at present the conventional method for *E. coli* detection requires several days, especially in cases where *E. coli* concentrations are low. Enrichment is a commonly used method for bacterial isolation to increase the cell counts of target bacteria. However, traditional culturing method is also a slow and laborious process requiring a series of steps and may require the use of adjunct methods (e.g. biochemical, serological, nucleic acid-based methods for conclusive identification) and can take up to a week for bacteria. The culture-based approaches coupled with other methods such as PCR, immunoassays, bacteriophages, NGS, Biosensors, and MALDI TOF MS are increasingly being used for the detection and identification of food borne pathogens. Nucleic acid-based methods such as Real-Time PCR and vPCR combined with sequencing approaches are more widely used than immunoassay and NGS-based approaches for pathogen detection. Nanobiotechnology is the latest approach for the detection of pathogens. Bacteriophages are one such unique biological entity that showed excellent host selectivity and have been actively used as recognition probes for pathogen detection. Antibiotics are efficient, powerful, and the most commonly used treatment against pathogenic *E. coli.* Other ways to treat or prevent *E. coli* contamination, such as probiotics, antimicrobial nanoparticles, and radiation treatment had been reported.

Keywords: *Escherichia coli;* ELISA; Enterohemorrhagic *E. coli* (EHEC); Detection methods; Food poisoning; PCR; India; Immunoassay; Total Coliform Count (TCC)

1. Introduction

Escherichia coli (*E. coli*) is a large group of a non-spore-forming, gram-negative bacteria usually motile by peritrichous flagella, and in most cases is harmless [1-36-167]. *Escherichia coli* (*E. coli*) naturally form the part of the normal flora in the gut of humans and other animals was first described by Theodor Escherich in 1885 [1-108-167]. Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora [1-167]. However, there are some

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strains that have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity [1-108-167]. These pathogenic *E. coli* can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors [1-108-167]. Among them, Enterohemorrhagic *E. coli* (EHEC) is defined as pathogenic *E. coli* strains that produce Shiga toxins (Stxs) and cause hemorrhagic colitis (HC) and the life-threatening Sequelae Hemolytic Uremic Syndrome (HUS) in humans [1-108-167]. Several serotypes in EHEC are frequently associated with human diseases such as O26:H11, O91:H21, O111:H8, O157:NM, and O157:H7 [1-108-167]. The Centers for Disease Control and Prevention (CDC), USA has estimated that *E. coli* O157:H7 infections cause 73,000 illnesses, 2,200 hospitalizations, and 60 deaths annually in the United States [1-108-167]. Several strategies for therapy have been studied including the use of antibiotics and vaccination [1-108-167]. However, there is no specific treatment for *E. coli* O157:H7 infection and the use of antibiotics may be contraindicated [1-108-167]. Therefore, treatment is mainly supportive to limit the duration of symptoms and prevent systemic complications [1-108-167]. Therefore, highly effective measures for prevention and control of *E. coli* O157:H7 infections are essential [1-108-167].

Food borne pathogens continue to pose a significant challenge to both food safety and global trade [1-56-167]. Food bacterial contamination caused not only human diseases but also serious economic damage [1-108-167]. The Public Health Agency of Canada has reported death from a salmonella outbreak involving cantaloupes, bringing the total to seven [157]. The agency says there have so far been 164 lab-confirmed cases of salmonella in eight provinces linked to Malichita and Rudy brand cantaloupes [157]. Quebec has been hardest hit, with 111 of those cases [157]. There have also been illnesses in British Columbia, Alberta, Ontario, Prince Edward Island, New Brunswick, Nova Scotia, and Newfoundland and Labrador [157]. The majority of people who became sick are children aged five and younger and adults aged 65 and older [157-167].

In India, food poisoning is common in settings where meals are prepared for large gatherings such as banquets, Langar, messes, religious occasions, and weddings [100-107]. Food borne diseases cause morbidity and mortality in the general population and they have emerged as a growing public health and economic problem in many countries during the last 2 decades [100-108]. Safeguarding public health during mass gatherings is a big challenge [1-100-107]. The important characteristics of such mass feeding occasions is that certain temporary arrangements are set up for cooking and serving food [1-108]. In these provisional kitchens, food safety measures from farm to table are difficult to implement and can lead to occurrence of food poisoning outbreaks [100-108-167]. In India, the need for a food safety law has been met by promulgation of Food Safety and Standards Act (FSSA) 2006, which applied to all eating establishments, messes, canteens, hospital kitchens, and religious places, where mass feeding takes place [100-107]. The symptoms of diarrhea, abdominal cramps, fever, and vomiting in a large number of cases after consumption of common food at a mass gathering confirmed the diagnosis of food poisoning [23-100-108]. In fact, the most *E. coli* are considered harmless to humans [1-66]. *Escherichia coli* serogroup O157 is the main causative agent of several intestinal and extra-intestinal food borne diseases in humans through consumption of low-dose contaminated foods such as milk, beef, and vegetables [1-70-107].

Food contamination by pathogenic microorganisms has been a serious public health problem and a cause of huge economic losses worldwide [1-108]. The Centre for Disease Control (CDC), USA has reported a consistent rise in the number of hospitalizations linked to food borne illnesses in the USA [1-76-108-167]. Food borne pathogenic *Escherichia coli* (*E. coli*) contamination, such as that with *E. coli* O157 and O104, is very common throughout the world even in India [1-60-167]. Six major food borne diarrheagenic *E. coli* Pathotypes: 1) Enteropathogenic *E. coli* (EPEC), 2) Shiga toxin-producing *E. coli*/enterohemorrhagic *E. coli* (STEC/ EHEC), 3) *Shigella*/Enteroinvasive *E. coli* (EIEC), 4) Enteroaggregative *E. coli* (EAEC), and 5) Enterotoxigenic *E. coli* (ETEC) and 6) diffusely adherent *E. coli* (DAEC) [1-76-108]. Infections due to *E. coli* (*Escherichia coli*) bacteria can cause severe, bloody diarrhea. Sometimes they also cause urinary tract infections, pneumonia, meningitis, bacteremia (a bacterial infection in the blood), or sepsis (a dangerous full-body response to bacteremia) [1-108-167]. Some infections can lead to serious health problems, especially in very young or very old people, or people with weak immune systems [1-108-167]. The bacteria can also spread from person to person on unwashed hands and surfaces, by swimming in contaminated water, and from touching animals at farms or petting zoos [1-108-167]. An outbreak is when a disease happens in greater numbers than expected in a particular area [1-108]. *E. coli* outbreaks usually happen because many people ate the same contaminated food [1-108-167].

Some types of *E. coli* bacteria make a toxin (a poisonous substance) that can damage the lining of the small intestine [1-108-167]. This can lead to bad stomach cramps, vomiting, dysentery and diarrhea (often with blood in it) [1-108]. Because of diarrhea, people can get dehydrated [1-108]. Symptoms usually start 3–4 days after a person had contact with the bacteria and end within about a week [1-108-167]. Most people recover completely from an *E. coli* infection [1-108-167]. But some can develop a serious kidney and blood problem called Hmolytic Uemic syndrome (HUS**)** [1-108-167]. Signs of HUS include: peeing less than usual, looking pale or swollen, unexplained bruises, bleeding from the nose or gums, extreme tiredness, and seizures [1-108]. HUS can be life-threatening and needs to be treated in a hospital [1-

108-167]. Kids with an *E. coli* infection should rest as much as possible and drink plenty of fluids to avoid dehydration [1-108-167]. Those who get dehydrated might need to get care in a hospital and be given IV fluids [1-108-167]. Kids with HUS may need dialysis for kidney failure and/or blood transfusions [1-108-167]. *E. coli* outbreaks have been tied to a wide variety of foods, such as fresh spinach, hamburgers, ground beef, bologna, hazelnuts, packaged cheeses, shredded lettuce, and prepackaged cookie dough [1-108-167].

Bacterial contamination may occur during one of the steps in the farm-to-table continuum from environmental, animal, or human sources and cause food borne illness [1-108-167]. *Escherichia coli* is an innocuous member of the human and warm-blooded animal gut microbiota [1-76-108-167]. However, pathogenic strains may cause intestinal and extra-intestinal infections [1-108-167]. *E. coli* is also one of the most important microorganisms used for the monitoring of water and food safety [1-36-108-167]. These primary hosts may acquire *E. coli* from water and food contaminated with feces. Therefore, the presence of *E. coli* is used as an indicator of fecal contamination [1-76-108-167]. Some *E. coli* strains have been isolated from various plants used for human consumption, and these plants, such as spinach, lettuce, alfalfa, cress, bean, arugula, tomato, and radish, are considered a secondary host [1-66-108-167]. Some strains of *Escherichia coli* (O157:H7 (STEC) are commonly associated with food poisoning outbreaks [1-66-108-167]. Among the bacteria associated with food borne illnesses are *Listeria monocytogenes*, *E. coli*, *Shigella soney*, *Salmonella*, and *Staphylococcus aureus* [1-56-108-167]. Over 700 strains or serotypes of *E. coli* exist in nature, water, and food [1-66-108-167]. Most *E. coli*—associated food borne outbreak cases over the past decade have been attributed to the consumption of uncooked foods contaminated by pathogenic *E. coli* at source and during the preparation process [1-66-108-167]. Food hygiene and safety are a major concern in the food industry, and microbiological safety is a particular problem [1-66-167]. *Escherichia coli* can act as an indicator for the presence of other pathogenic bacteria, and it is detected easily in foods such as pork, beef, chicken and vegetables [1-23-108-167]. Thus, *E. coli* detection in foods is one of the most useful hygienic criteria [1-76-108-167].

E. coli possesses quite a few virulence factors encoded on mobile genetic elements and/or plasmids or localized in pathogenicity islands [1-66-108-167]. These virulence factors are expressed as endotoxins, exotoxins, adhesion, invasion, or iron acquisition factors [1-108-167]. However, the number of food borne outbreaks associated with fresh produce has been increasing, with *Escherichia coli* being the most common pathogen associated with them [1-66-108-167]. In humans, pathogenic *E. coli* strains cause diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and other indications [1-56-108-167]. There are current reports on outbreaks caused by the consumption of lettuce irrigated with water contaminated with *E. coli* O157 : H7[1-108-167]. Further, *STEC E. coli O157 : H7.* Verotoxigenic or Shiga-like toxigenic *E. coli* (VTEC or STEC) O157 : H7 is considered a large threat in food borne diseases [1-108-167]. *E. coli* O157 : H7 became the first of several strains referred to as Enterohaemorrhagic *E. coli* or EHEC, which can produce one or more Shiga toxin (also called verocytotoxins and formerly known as Shiga-like toxins) [1-76-108-167]. STEC strains can survive in fresh ground beef and on fresh leafy green vegetables, and it is well known that the main reservoirs for VTEC are ruminants, which continually shed bacteria into the environment, contaminating food and water [1-76-108-167]. Vegetables can be contaminated with *E. coli* at any point from pre- to postharvest [1-56-108-167]. This bacterium, *E. coli* is able to survive in many environmental conditions due to a variety of mechanisms, such as adhesion to surfaces and internalization in fresh products, thereby limiting the usefulness of conventional processing and chemical sanitizing methods used by the food industry [1-66-108-167]. There have been many studies to develop novel antimicrobial drugs and vaccines against pathogenic *E. coli* and disease symptoms [1-66-108-167]. However, drug therapy and antimicrobial substances applied to the environment and food are only a temporary solution [1-76-108]. The quality improvement of environmental sanitation and personal hygiene may be the best way to prevent pathogenic *E. coli* infection and food borne outbreak [1-76-108-167]. In the following section, *E. coli* symptoms, pathogenesis, detection methods and controlling measures have been updated and discussed.

1.1. *Escherichia coli* (*E. coli*)

Escherichia coli is a rod-shaped bacterium and it is a facultative anaerobe that swiftly colonizes the gastrointestinal tracts of both humans and animals shortly after birth, benefiting both the host and bacterium [1-23-108]. It belongs to the family *Enterobacteriaceae* and falls under the *Escherichia* genus. When grown under aerobic conditions at a temperature of 37° C, it exhibits robust growth on both general and selective agar media [1-23-167]. This growth results in the formation of distinct round colonies [1-23-108-167]. *E. coli* is typically oxidase-negative, catalase-positive, capable of reducing nitrate to nitrite, showed motility, lacks acid-fast properties, and does not form spores [1-23-108]. The identification of specific strains of *E. coli* has traditionally relied on serotyping, a method that involves characterizing the presence of O (somatic), H (flagellar), K (capsular), and F (fimbriae) antigens [1-23-108-167]. In the late 19th century, pediatrician Theodore *Escherich* discovered *E. coli* in 1885 and initially referred to it as normal intestinal flora, naming it "Bacterium coli commune" [1-23-108]. Later, it was officially renamed as *E. coli*. This bacterium belongs to the *Enterobacteriaceae* family and exhibits facultative anaerobic characteristics [1-23-108-167].

E. coli can be motile, often utilizing flagella, but can also be non-motile, and it can thrive in both aerobic and anaerobic environments [1-23-108]. *Escherichia coli* is one of the most frequently encountered bacteria in clinical samples [1-23-108]. In healthy hosts, the most *E. coli* strains are non-pathogenic and contribute significantly to the commensal population residing in the host's intestinal tract, primarily within the mucosal layer of the colon [1-23-108]. However, *E. coli* can become pathogenic under certain circumstances, such as when the host's immune system is suppressed [1-23-108]. Some strains are inherently pathogenic and can cause gastrointestinal and urinary tract infections [1-108-167]. *Escherichia coli* can endure for extended periods in environmental settings like soil and water [1-23-108]. The presence of *E. coli* in food or water can signal inadequate cleaning and careless handling, or it may suggest the potential presence of enteric pathogens [1-23-108-167]. Based on genetic and clinical criteria, *E. coli* can be broadly classified into three major groups: Commensal *E. coli*, intestinal pathogenic (diarrheagenic) *E. coli*, and extra-intestinal pathogenic *E. coli* (ExPEC) [1-23-108-167]. Additionally, various molecular typing techniques, including PCR (polymerase chain reaction) and PFGE (pulsed-field gel electrophoresis), can be employed to differentiate between *E. coli* strains [1-23-108].

The polymerase chain reaction (PCR) has become a cornerstone technique in molecular biology and microbiology for its ability to selectively amplify specific DNA sequences. It is extensively utilized in the detection of genes and their variants within various organisms, including *E. coli* [1-23-108-167]. However, the absence of a PCR signal for a particular gene of interest in *E. coli* isolates can pose challenges and uncertainties [1-23-108-167]. It is crucial to explore the reasons behind such failures and consider the implications they may have on research outcomes and interpretations [1-23-108-167].

Most *E. coli* found in the environment are non-pathogenic [1-23-108]. However, some groups are pathogenic. Generally, pathogenic *E. coli* is broadly classified into two major categories: diarrheagenic *E. coli* and extra intestinal pathogenic *E. coli* [1-23-108-167]. The intestinal or diarrheagenic pathogenic strains of *E. coli* are rarely found among the intestinal flora of healthy mammals [1-23-108]. Based on the virulence factors, six different pathogenic classes of intestinal pathogenic *E. coli* have been identified, namely, Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) [1-23-108-167].

1.2. *E. coli* Infection Symptoms

The pathogenic *E. coli* strains can infect the gut area and cause severe illness [1-2-108-167]. *Escherichia coli* (*E. coli*) bacteria normally live in the intestines of healthy people and animals [1-2-108]. Most types of *E. coli* are harmless or cause relatively brief diarrhea [1-108-167]. But a few strains, such as *E. coli* O157:H7, can cause severe stomach cramps, bloody diarrhea and vomiting [1-2-108]. Exposure to *E. coli* infection from contaminated water or food especially raw vegetables and undercooked ground beef [1-2-108-167]. Healthy adults usually recover from infection with *E. coli* O157:H7 within a week [1-2-108]. Young children and older adults have a greater risk of developing a life-threatening form of kidney failure [1-2-108-167]. Signs and symptoms of *E. coli* O157:H7 infection usually begin three or four days after exposure to the bacteria [1-2-108-167]. But infected person may become ill as soon as one day after exposure to more than a week later [1-2-108-167]. Signs and symptoms include: diarrhea, which may range from mild and watery to severe and bloody [1-2-108-167]. Further severe stomach cramping and tenderness, pain or tenderness, nausea, vomiting, fever, bleeding, seizures, headache, confusion, kidney failure, constant fatigue in some people [1-2-108]. Furthermore, only a few strains of *E. coli* trigger diarrhea. The *E. coli* O157:H7 strain belongs to a group of *E. coli* that produces a powerful toxin that damages the lining of the small intestine [1-2-108-167]. This can cause bloody diarrhea [1-2-108]. Person may develop an *E. coli* infection when ingest this strain of bacteria [1-2-108]. Unlike many other disease-causing bacteria, *E. coli* can cause an infection even if person ingested only small amount of contaminated food or water [1-2-108]. Because of this, person can be sickened by *E. coli* from eating a slightly undercooked hamburger or from swallowing a mouthful of contaminated pool water [1-2-108-167].

Human and animal stool may pollute ground and surface water, including streams, rivers, lakes and water used to irrigate crops [1-2-108-167]. Although public water systems use chlorine, ultraviolet light or ozone to kill *E. coli*, but some *E. coli* outbreaks have been linked to contaminated municipal water supplies [1-2-108-167]. Rural water supplies are the most likely to be contaminated [1-2-108]. Some people also have been infected with *E. coli* after swimming in pools or lakes contaminated with stool [1-2-108]. Another source of infection might be unpasteurized milk [1-2-108-167]. *E. coli* bacteria on a cow's udder or on milking equipment can get into raw milk. [1-2-108]. Runoff from cattle farms can contaminate fields where fresh produce is grown. Certain vegetables, such as spinach and lettuce, are particularly vulnerable to this type of contamination [1-2-108-167]. Ground chicken and beef is another source of contamination [1-2-108-167]. When chicken are slaughtered and processed, *E. coli* bacteria in their intestines can get on the meat [1-2-108]. Ground beef combines meat from many different animals, increasing the risk of contamination

[1-2-108-167]. *E. coli* bacteria can easily travel from person to person, especially when infected adults and children do not wash their hands properly [1-2-108]. Family members of young children with *E. coli* infection are especially likely to get it themselves [1-2-108]. Outbreaks have also been occurred among children visiting petting zoos and in animal barns at county fairs [1-2-108-167]. Most healthy adults recovered from *E. coli* illness within a week [1-2-108]. Some people particularly young children and older adults may develop a life-threatening form of kidney failure called hemolytic uremic syndrome (HUS) [1-2-108]. In fact, 75 to 95% of urinary tract infections are caused by *E. coli*. Further, *E. coli* is a normal resident of the bowel, which is how it makes it way to the urinary tract. Some versions of *E. coli* make sick by making a toxin called Shiga and called as STEC (Shiga Toxin Producing *E. coli*) [1-2-108-167]. This toxin damages the lining of the intestine. With some strains of *E. coli*, severe blood and kidney problems may occur within 2 weeks after the diarrhea [1-2-108-160]. These problems can cause kidney failure and sometimes long-term disability or death in some children and older adults [1-2-108-165]. When *E. coli* causes serious problems with the blood or kidneys, symptoms include: pale skin, a fever, weakness, bruising, and passing only small amounts of urine [1-2-108-160].

Vomiting usually happens earlier on the disease, diarrhea usually lasts for a few days, but can be longer depending on the organism that is causing the symptoms [1-2-108-150]. Conversely, a person is more likely to have diarrhea with mucus and blood in bacterial diarrhea [1-2-108-160]. One of the dangers of food poisoning and gastroenteritis especially in very young, old, or otherwise vulnerable people is the loss of fluids Trusted Source resulting from diarrhea and vomiting, which can lead to dehydration [1-2-108-156]. Dehydration can, however, be prevented. In case of parasitic gastroenteritis, another danger is malnutrition [1-2-108-167]. The parasites reach the intestines and feed off the nutrients which person absorbs from their food [1-2-108]. This results in a person developing a chronic lack of nutrients [1-2-108-167]. To avoid the dangerous and potentially fatal effects of dehydration from diarrhea, a person should drink oral rehydration salts (ORS) [1-2-108]. Dehydration has been a more significant risk in low or middle-income countries [1-2-108-160]. In higher-income countries, while the threat of death is smaller, rehydration is nonetheless important [1-2-108]. A person can replace salt, glucose, and minerals lost through dehydration through sachets of oral rehydration salts available from pharmacies and online[1-2-108]. A person can dissolve the salts in drinking water and this does not required a doctor's prescription [1-2-108]. It is important to get the right concentration, as too much sugar can make diarrhea worse, while too much salt can be extremely harmful, especially for children [1-2-108]. A more diluted solution (for instance using more than 1 liter of water), is preferable to a more concentrated solution [1-2-108].

An *E. coli* infection usually goes away on its own. The main treatment is to make patient comfortable and drink sips of water [1-2-108]. Diarrhea causes the body to lose more water than usual. This can lead to dehydration, which is especially dangerous for babies and older adults [1-2-108]. Taking frequent, small sips of water will help to prevent dehydration [1-2-108]. *E. coli* usually goes away on its own and most of the time, person do not need antibiotics [1-2-108]. If a person have diarrhea, do not use over-the-counter anti-diarrheal medicine, such as Imodium or Kaopectate Anti-Diarrheal [1-2-108]. When person feels like eating again, start with small amounts of food. To prevent dehydration, drink plenty of fluids [1-2-108]. Choose water and other clear liquids [1-2-108]. Soda, fruit juices, and sports drinks have too much sugar and not enough of the important electrolytes that are lost during diarrhea [1-2-108]. These kinds of drinks should not be used to rehydrate. If patient have kidney, heart, or liver disease and have to limit fluids, and immediately consult family doctor before increasing the intake of amount of fluids [1-25-108]. Wash hands often and always wash them after bowel movements. If patient have bloody diarrhea that may be from an *E. coli* infection, do not take diarrhea medicine [1-2-108]. These medicines can slow down the digestion process [1-100-108]. This can allow more time for body to absorb the poisons made by the *E. coli* [1-2-108]. In some people, the infection causes serious problems with the blood and kidneys [1-2-108]. These people may need blood transfusions or dialysis [1-23-108].

Pathogenic *E. coli* infection usually causes severe diarrhea. *Escherichia coli* is the most common cause of acute urinary tract infections as well as urinary tract sepsis [1-2-100]. It has also been known to cause neonatal meningitis and sepsis and also abscesses in a number of organ systems [1-27-108]. *Escherichia coli* may also cause acute enteritis in humans as well as animals and is a general cause of 'Traveller's diarrhoea', a dysentery-like disease affecting humans, and haemorrhagic colitis often referred to as 'bloody diarrhoea'[2-108]. A list of some of the strains of *E. coli* that can cause a number of illnesses. Virulence types of *E. coli* include Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enteropathogenic (EPEC), and Vero cytotoxigenic (VTEC) [1-29-100]. Moreover, *E. coli* are introduced into water bacteriology because it is a useful marker of faecal pollution and thus became an important marker in food and water hygiene [2-108]. The persistence of *E. coli* in natural environments will undoubtedly be aided through its ability to grow as a biofilm [2-108]. For *E. coli* 0157, the levels of chlorine generally found in water have been shown to be sufficient for its inactivation [1-108]. The World Health Organization (WHO) has reported that the Middle East and North Africa (MENA) region ranks as the third highest in terms of the burden of food borne diseases per population, following closely behind the Southeast Asian and African regions [1-23-108].

Pathogenic *E. coli* not only caused huge economic losses as a result of these cases but also impacted human health and even caused death [2-108]. Diarrhea is the result of the reversal of the normal net absorptive status of water and electrolyte absorption to secretion [1-2-108]. Worldwide, there are nearly 2. 5 billion cases of diarrheal disease every year. Diarrheal disease is the second leading cause of death in children under 5 years old [1-2-108]. Every year about 900,000 children under 5 years old die due to diarrheal diseases [1-2-108]. Clearly, pathogenic *E. coli* food borne outbreak is still a significant cause of human illness worldwide [1-23-108]. In Europe, food borne infections have emerged as a prominent public health concern [23-108]. Reports from the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) have designated Salmonella, Campylobacter, Listeria, and Shiga toxin-producing *E. coli* as high-priority pathogens at the European Union (EU) level [1-23-109].

Fortunately, diarrheal disease caused by pathogenic *E. coli* is preventable by improved environmental sanitation and is treatable by antibiotics [1-2-100]. The treatment of diarrheal disease is generally effective with oral rehydration and maintaining electrolyte balance through the diet [1-2-108]. Patients with severe dehydration may required intravenous rehydration and use of anti-diarrheal, pain relief drugs and antibiotics can slow down the patient's symptoms [1-2-108]. Hospitalization and fatality rates of *E. coli* O157: H7 are significantly higher than that of other *E. coli* serogroups and food borne pathogens including *Campylobacter* and *Salmonella* [1-2-108]. The infectious dose of this food borne pathogen is 10–100 bacterial cells, leading to different intestinal or extra-intestinal diseases, which might progress into the stages that threaten human life [1-23-167]. Quantitative prevalence evaluation of different food samples has been reported as one of the most efficient strategies to control and prevent any outbreak caused by this pathogen [1-23-108-167].

Diarrheagenic *E. coli* pathotypes can be passed in the feces of humans and other animals [1-2-108]. Transmission occurs through the fecal–oral route, via consumption of contaminated food or water, and through person-to-person contact, contact with animals or their environment, and swimming in untreated water [1-23-108-167]. Humans constitute the main reservoir for non-STEC pathotypes that cause diarrhea in humans [1-23-108]. The WHO report also indicated that approximately 70% of food borne diseases in the Middle East and North Africa region are attributed to *E. coli*, Campylobacter, non-typhoidal Salmonella (NTS), and Norovirus, underscoring the significant threat posed by these disease-causing agents [1-23-108]. The intestinal tracts of animals, especially cattle and other ruminants, are the primary reservoirs of STEC [1-23-108]. However, certain strains that can cause infections and illness become resistant to antibiotics [1-2-108]. The pathogenic *E. coli* can be found in soil and water, usually as a result of animal fecal contamination [1-23-108]. Several pathotypes of *E. coli* have been reported and cause infectious diseases in humans [1-23-108-167]. Unsafe food also poses major economic risks, especially in a globalized world. According to the WHO website, the *E. coli* O104:H4 outbreak caused a loss of US$1.3 billion to Germany's farmers and industries and required payments of US$236 million in emergency aid to 22 European Union member states in 2011 [1-23-108].

1.3. *E. coli* Food Poisoning in India

Food-borne transmission of pathogenic and toxigenic microorganisms has been a recognized as hazard for decades [1-23-108]. Food borne diseases are one of the health hazards and causes of morbidity and mortality in developing countries [1-100-108-167]. Under the Integrated Disease Surveillance Project (IDSP) in India, food poisoning outbreaks are reported from all over the country [100-108]. Out of the total outbreaks reported to the IDSP, approximately 60% are related to food-borne infections [1-100-108]. Food poisoning outbreaks are commonly seen in mass social events where food is prepared under temporary arrangements [100-108]. One of the study reported a food poisoning outbreak in a city of western Maharashtra, India, where around 4000 people had consumed food during a religious community lunch and reported sick to the nearby hospital with complaints of diarrhea, abdominal cramps, fever with chills, and vomiting [100-108]. Investigation of the food poisoning outbreak was conducted and identified the causes and recommended preventive measures [100-108]. Interview method was used to elicit food history from the affected and non-affected persons [100-108]. Inspection of the cooking area was conducted to find the likely source of contamination [100-108]. A total of 291 patients reported sick after consumption of meal at a religious mass gathering [100-108]. The range of incubation period was from 10 hours to 40 hours. Predominant features were diarrhea (100%), abdominal cramps (89%), fever with chills (81%), and vomiting (28.5%) were reported [100-108]. Maximum relative risk of 14.89 was seen for green gram (moong dal**)** with 95% confidence interval of 2.16-102.6 [100-108]. Keeping the incubation period and clinical profile in view, the likely organisms are Enteropathogenic *Escherichia coli* or Salmonella spp [100-108]. Maintaining food safety during mass gatherings is a major challenge for public health authorities [100-108]. The Food Safety and Standards Act (2006) in India brings the food consumed during religious gatherings such as 'prasad' and 'langar' under its purview and comprehensively addresses this issue [100-108].

Prevention of food poisoning during mass gatherings involves stringent hygiene standards and safety surroundings while preparing food [100-108]. Food warmers should be used to store the cooked food above 70°C to prevent growth

of microorganisms [100-108]. Consumption of uncooked foods such as salads, fruits, and raw milk should ideally be avoided [100-108]. Food samples of food items prepared should be preserved for 72 h in deep freezers to aid an investigation in case of any food poisoning outbreaks [100-108]. Food borne illnesses are leading source of morbidity and mortality in both developed and developing nations[1-100-108]. *Escherichia coli* O157 is one of the most reported food borne pathogen that emerged in the past few decades [1-100-108]. South East Asia region suffers the highest average burden of diarrhoeal mortality, especially when it comes to child mortality [1-100-108]. Many studies were undertaken in the developed nations and evaluated the role of *E. coli* O157 as one of the etiological agent in food borne outbreaks [1-100-108]. The distribution of *E. coli* O157 serotype in the food chains of South East Asian countries, with a special focus on India where more than half a million child diarrhoeal deaths occurs every year and the reasons for which is often not ascertained to the fullest extent [100-108].

Food is an integral part of all social events. Such events expose masses to risk of food-borne infections as the food is prepared under temporary arrangements [100-108]. The application of WHO Five Keys to Safer Food can prevent such occurrences [1-100-108]. The Food Safety and Standards Authority (FSSA) in India is a forward-looking act aimed at food safety at all levels [100-108]. In India, majority of outbreaks of food borne disease go unreported, unrecognized or un-investigated and may only be noticed after major health or economic damage has occurred [100-108]. In such a condition controlling the outbreaks, detection and removal of implicated foods, identification of the factors that contribute to the contamination, growth, survival and dissemination of the suspected agent, prevention of future outbreaks, strengthening of food safety policies and programmes is not possible [100-108]. The total count of bacteria per gram of food is a good guide to the hygiene and temperature control process in the preparation of food [100-108]. The higher the number of bacteria, the greater is the risk that food poisoning organisms may be present in the food [100-108]. Recorded food poisoning outbreaks are very few in India [100-108]. The reported bacterial food borne disease outbreaks in India during 1980-2009 indicated that 24 outbreaks have occurred involving 1,130 persons [100-108]. It is observed that the important bacterial agents, which caused these outbreaks were Staphylococcus aureus, Vibrio, Salmonella, *E. coli* and Yersinia enterocolitica [100-108].

In India, there are no systematic studies to understand the types of foods involved and the etiological agent causing the disease [100-108]. Therefore, a pilot study was proposed and investigated the food poisoning cases, undertaken by the Ronald Ross Institute of Tropical Diseases, which is a referral hospital for food borne diseases in Hyderabad [100-108]. During this study, food and stool/rectal swabs of the patients affected were collected for microbiological examination [100-108]. Epidemiological, environmental, and laboratory components indicated that *Staphylococcus aureus* was the etiological agent in most of the cases and in one case Salmonella spp and *E. coli* were the main cause of food poisoning [100-108]. This study also indicated the need to take up food borne disease surveillance under the Indian context and identified the common high-risk food commodities for microbial contamination and identification [100-108]. Food poisoning is a major cause of gastroenteritis [100-108]. Drinking plenty of fluids, resting, and gradually increasing food intake can help people to recover from gastroenteritis due to food poisoning [1-100-108].

1.4. *E. coli* contamination of leafy vegetables

It has been shown that how crops are harvested, processed, and distributed has enhanced both the supply and variety of products, which may also have increased the risk of more widespread outbreaks [1-100-108-160]. The increase in illness associated with consumption of fresh produce reflects a documented increase in food contamination [1-100-108]. Food borne illness may be the cause of fresh produce contamination by pathogenic bacteria, viruses, and protozoa [1-100-108-165]. This contamination may originate from manure, soil, sewage, surface water, or wildlife [1-100-108]. It may also occur during washing, slicing, soaking, packing, and food preparation [1-100-108]. Among the bacteria associated with food borne illnesses are *Listeria monocytogenes*, *E. col*, *Shigella soney*, *Salmonella*, and *Staphylococcus aureus* [1-100-108].

The presence of enteropathogenic bacteria in fresh produce plays an important role in the emergence of food borne outbreaks [1-100-108-167]. There are many possible sources of contamination on fresh produce due to exposure to many different environments and handling [1-100-108]. More studies are necessary to better understand how to prevent the occurrence of *E. coli* on fresh produce [1-100-108-167]. The attachment to plant surfaces is the first step in the colonization process and subsequent transmission of pathogens via the edible parts of plants [1-100-108]. However, each enteropathogen has its own molecular mechanisms of adherence and fitness to the vegetable biosphere; many are similar to mechanisms used to colonize the primary host [1-100-108]. All enteropathogens survived in fresh produce for commercially relevant periods despite the use of multiple disinfection systems [1-100-108-167]. The future of food safety lies in adherence to strategies for the different categories of *E. coli* pathogens [1-100-108]. These measures will help to prevent bacterial transmission and benefit human health [1-100-108]. Finally, growers, producers, packers, and food consumers need to examine their own processes and incorporate strategies for maintaining food safety [1-100-

108]. Survival and growth of these microorganisms depend on several factors, including the specific features of the microorganism, fruit ripeness, environmental conditions, plant development, bacterial resistance to the plant metabolic processes, plus harvest, and postharvest processes [1-100-108-167]. Particularly, some pathogenic microorganisms can internalize and adhere to the plant surface [1-100-108].

1.5. *E. coli* Pathogenesis

The five major food borne diarrheagenic *E. coli* pathotypes: 1) Enteropathogenic *E. coli* (EPEC), 2) Shiga toxin-producing *E. coli*/ Enterohemorrhagic *E. coli* (STEC/EHEC), 3) *Shigella*/enteroinvasive *E. coli* (EIEC), 4) Enteroaggregative *E. coli* (EAEC), and 5) Enterotoxigenic *E. coli* (ETEC) [1-100-108].

1) **Enteropathogenic *E. coli* (EPEC):** Enteropathogenic *Escherichia coli* (EPEC) are important diarrheal pathogens in children. In 1945, the EPEC strains were the first diarrheagenic *E. coli* to be identified during the outbreaks of infantile diarrhea in the United Kingdom [1-100-108]. EPEC are highly prevalent in community settings (such as schools and hospitals) and are a main cause of persistent diarrhea [1-100-108]. The most common symptoms of EPEC illness are watery diarrhea, abdominal pain, nausea, vomiting, and fever [1-100-108]. In addition to humans, EPEC can also infect animals such as cattle, dogs, cats, and rabbits [1-100-108]. In most cases, EPEC-induced diarrhea is self limiting and can be effectively treated with oral rehydration therapy [1-100-108]. Persistent infections may required the use of antimicrobials. However, resistance to various agents has been reported [1-100-108]. A hallmark phenotype of EPEC is the ability to produce attaching and effacing (A/E) lesions on the surfaces of intestinal epithelial cells [1-100-108].

2) Shiga‑toxin‑producing *E. coli* (STEC): *Escherichia coli* carrying the *stx* gene to produce Shiga toxins (Stxs) are defined as Shiga-toxin-producing *E. coli* (STEC), also known as verocytotoxin-producing *E. coli* (VTEC) [1-100-108]. Strains of STEC can cause hemorrhagic colitis (HC) and are commonly referred to as Enterohaemorrhagic *E. coli* (EHEC) [1-100-108]. Transmission of STEC infection mainly occurs through eating or handling contaminated food and coming into contact with infected animals [1-100-108]. Further, person-to-person transmission is possible by close contact such as within families or at schools and in nursing homes [1-100-108]. STEC infections are mostly food borne, and a variety of food sources such as undercooked ground beef, raw milk, salad, raw leaves and potatoes, vegetables, fruits, and other foods are implicated in outbreaks [1-100-108]. Many raw vegetables, such as tomato, chili, onion, lettuce, arugula, spinach, and cilantro, are incorporated into fresh dishes including ready-to-eat salads and sauces [1-100-108].The consumption of these foods confers a high nutritional value to the human diet [1-100-108]. However, the number of food borne outbreaks associated with fresh produce has been increasing, with *Escherichia coli* being the most common pathogen associated with them [1-100-108]. In humans, pathogenic *E. coli* strains cause diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and other indications [1-100-108]. Vegetables can be contaminated with *E. coli* at any point from pre- to postharvest [1-100-108]. This bacterium is able to survive in many environmental conditions due to a variety of mechanisms, such as adhesion to surfaces and internalization in fresh products, thereby limiting the usefulness of conventional processing and chemical sanitizing methods used by the food industry[1-100-108].

The symptoms of STEC infection in humans are watery diarrhea, HC, hemolytic uremic syndrome (HUS), fever, abdominal cramping, and vomiting [1-100-108]. General treatment is oral rehydration therapy and antibiotic therapy [1-100-108]. There are many serotypes of STEC such as O26, O111, O121, and O157 in which serotype O157:H7 is most often implicated in foodborne-illness outbreaks in the world [1-100-108].

3) *Shigella*/enteroinvasive *E. coli* (EIEC): Enteroinvasive *E. coli* (EIEC) and *Shigella* strains have the ability to invade the human mucosa of the colon, M cells, macrophages, and the epithelial cells [1-100-108]. EIEC is closely related to *Shigella* spp. and causes bacillary dysentery (also called shigellosis) in humans [1-100-108]. The symptoms of *Shigella*/EIEC infection range from mild watery diarrhea to severe inflammatory bacillary dysentery characterized by strong abdominal cramps, fever, chills, and stools containing blood and mucus [1-100-108]. Severe symptoms can even be fatal and severe life-threatening complications, including megacolon, intestinal perforation, peritonitis, pneumonia, and HUS, can occur [1-100-108].

4) Enteroaggregative *E. coli* (EAEC): EAEC strains are important causative agents of traveler's diarrhea and cause persistent diarrhea in immunocompromised older people and children in developing countries [1-100-108]. The symptoms of EAEC infection are often watery diarrhea with mucus and are accompanied by fever, vomiting, and abdominal pain [1-100-108]. The EAEC infection treatments are oral rehydration therapy and antimicrobial therapy[1-100-108]. Antibiotics are useful for treating cases of traveler's diarrhea [1-100-108]. However, antibiotic resistance of EAEC is increasing worldwide [1-100-108].

5) Enterotoxigenic *E. coli* (ETEC): In developing countries and semitropical areas such as Latin America, the Caribbean, Southern Asia, and Africa, Enterotoxigenic *E. coli* (ETEC) are a major cause of traveler's diarrhea and the childhood diarrhea pathogen [1-100-108]. The typical clinical symptoms of ETEC infection are often watery diarrhea, abdominal pain, nausea, vomiting, and fever [1-100-108]. The symptoms will last about 3–5 days [1-100-108]. ETEC infection is acquired by ingestion of contaminated food or water [1-100-108]. Due to the high infectious dose, ETEC are spread by contaminated food and water, not by human-to-human transmission [1-100-108]. Oral rehydration therapy and antibiotic therapy are very effective for ETEC infection diarrhea [1-100-108]. In recent years, many reports have indicated that antimicrobial resistant ETEC strains are increasing worldwide [1-100-108]. Bacterial contamination may occur during any of the steps in the farm-to-table continuum from environmental, animal, or human sources and cause food borne illness [1-100-108]. In the past 20 years, food borne-illness outbreaks and cases associated with fresh produce have rapidly increased [1-100-108]. Over the past 10 years, food borne outbreaks were chiefly caused by EPEC, STEC/EHEC, EIEC/*Shigella*, ETEC, and EAEC [1-100-108]. Bacterial contamination may occur during any of the steps of the farm-to-table continuum [1-100-108]. Contaminated foods can be divided into fruits, vegetables such as raw clover sprouts, romaine lettuce, sprouts, cucumbers, raw leeks, potatoes, spinach, basil pesto, lettuce and fresh basil; meat and meat products such as chicken salad, ground beef, raw beef dishes, raw pre-packaged cookie dough, chives and scrambled eggs, kimchi, cheese, cooked food (due to the food handler) such as egg soup and tuna bibimbap [1-100-108].

1.6. How to control *E. coli* Pathogenesis

Food borne or waterborne disease occurs when a food borne or waterborne pathogen is ingested with food or water and established itself in the human or animal host [1-100-108-167]. Food borne diseases are a great threat to public health and be regarded as the main concern and challenge in food industry, health, and hand safety [1-100-108]. It may also occur when a toxigenic food borne or waterborne pathogen established itself in a food or water supply and produces different intestinal and extra-intestinal toxins which are ingested [1-100-108-167]. More than 250 different food borne hazards have been recognized. Notably, most severe food borne cases occur in the very old, the very young, pregnant, and immune compromised individuals [1-100-108-167]. The World Health Organization declared that more than 1.5 million people died from the acute diarrheal diseases caused by different food borne pathogens around the world Food borne diseases are a great threat to public health and be regarded as the main concern and challenge in food industry, health, and hand safety [1-100-108-160]. The most common food borne bacterial pathogens are pathogenic *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Shigella* spp., and *Cronobacter sakazakii* [1-100-108].

Escherichia coli is a rod-shaped, Gram-negative, facultative anaerobe, non-sporulating bacterium belonging to the *Enterobacteriaceae* family, which is part of the normal gastrointestinal microbiota of humans and animals [1-100-108]. It is also commonly found in abundance in soil and water. Different serotypes of *E. coli* have been identified and isolated from clinical, food, water, and environmental samples [1-100-108]. ***Escherichia coli* O157** has been known as the most common serotype of pathogenic *E. coli* strains identified as enterohemorrhagic and Shiga-toxin producing bacterial food borne pathogens [1-100-108].

Antibiotics are efficient, powerful, and the most commonly used treatment against pathogenic *E. coli* in clinical and animal agriculture [1-100-108]. However, large numbers of drug-resistant strains have appeared as the result of overuse of antibiotics in the past 50 years [1-100-108]. Antibiotic treatment also brings an increased risk of the symptoms. Several antibiotics such as ampicillin cotrimoxazole, trimethoprim, azithromycin, and gentamicin for combating pathogenic *E. coli* have been shown to stimulate Stx release from *E. coli* [1-100-108-167]. In addition to the use of antibiotics, antibody therapy is another method for neutralizing virulence factors and toxins from pathogenic *E. coli* and reducing symptoms. Combining antibiotic and antibody is a promising strategy for future STEC treatments [1-100-108-167]. The anti-virulence factor antibody development could help to improve detection of STEC in livestock, food, the environment, and in clinical samples, resulting in an improved food safety and human health [1-100-108]. Many studies have indicated the feasibility of *E. coli* vaccines [1-100-108]. The *E. coli* vaccine development usually used attenuated *E. coli*, inactivated whole *E. coli*, virulence factors, and toxins to induce immune responses in humans [1-100-108]. The disadvantage of vaccines is that effective duration of the immunity is short. There is still no vaccine available to effectively control the spread of pathogens *E. coli* [1-100-108-160].

Another method to control the bacterial infections is the use of Bacteriocins. Bacteriocins are natural food additives due to the bacteriocin-producing bacteria presence in many types of foods [13-100-108-167]. Therefore, bacteriocin producing bacteria or bacteriocins can be applied on the food against food-spoiling bacteria and food-borne pathogens [1-100-108-167]. At present, only a few bacteriocins are allowed to be used in food or feed and are not treated as a therapeutic drug [1-100-108]. On the other hand, the *E. coli* can obtain the different bacteriocin immunity genes by conjugation because the bacteriocin to be ineffective [1-100-108]. More than 99% of bacteria can produce at least one

bacteriocin, most of which are not identified. Bacteriophages (phage) are bacterial viruses (viruses that infect bacteria) that can be found in all natural environments such as animal feces, waste water, and soil [1-100-108-167]. In animal agriculture, phages can be applied to control diseases caused by pathogenic *E. coli* [1-100-108-160]. Plant extract additives not only act as potential natural and safe antimicrobial alternatives but also as spices and flavouring agents to improve the texture of cheese. The plant extracts used in cheese may serve as antibacterial agents against serious food borne pathogens such as *E. coli* O157:H7 *Listeria monocytogenes, Salmonella* Typhimurium, and *Staphylococcus aureus* [1-100-108-167]. It was also shown that the combination of plant material and antibiotic could improve the antibiotic activity against multidrug-resistant *E. coli* [1-100-108-167].

Other ways to treat or prevent *E. coli* contamination, such as probiotics, antimicrobial nanoparticles, and radiation treatment had been reported [1-100-108]. In daily food, continuous supply of probiotics could help humans and livestock to face pathogenic microorganisms [1-100-108]. Probiotics can secrete anti-bacterial or anti-virulent agents to act against pathogenic *E. coli* [1-100-108]. Antimicrobial nanoparticles could be applied on vacuum-packaged meat and poultry products to control the food borne pathogens *E. coli* O157:H7 [1-100-108]. Drugs are available to reduce pathogenic *E. coli*: Antidiarrheal medication such as loperamide (branded versions include Imodium, and Imotil, among others) and bismuth subsalicylate (Pepto-Bismol). Further antiemetic (anti-vomiting) medication such as chlorpromazine (Thorazine) and metoclopramide (Reglan and Metozolv) [1-100-108]. Antiparasitic medications such as Metronidazole (Flagyl) or Ivermectin (Stromectol) (The exact medication will depend Trusted Source on the type of parasite). A person should speak with a doctor before taking anti-diarrhea medication as some infections may get worsened with anti-diarrhea medicines [1-100-108]. According to some newer research, probiotics (live "good" bacteria and yeasts) may also be helpful in treating gastroenteritis [1-100-108]. One study found that the use of probiotics in children hospitalized for acute gastroenteritis shortened the duration of diarrhea by a mean of 1.16 days [1-100-108]. In some cases, stool testing is necessary [1-100-108]. For example, if diarrhea is accompanied by blood or is watery for more than a few days, doctors may want a stool sample to test for parasites or bacteria [1-100-108].

Food borne infections originating from bacterial pathogens like pathogenic *E. coli* are a prevalent cause of human illnesses in the throughout the world, leading to significant economic losses and public health consequences [1-100-108]. These *E. coli* pathogens' genetic material is frequently found in various food items across many countries. The existing evidence highlighted that the identification of these bacterial pathogens is common in animal based food products [1-100-108]. In contrast, when it comes to fruits and vegetables, the available data on these pathogens are limited compared to animal-derived foods [1-100-108]. These bacteria can enter the human food supply chain from their initial production stages to the final consumption of products [1-100-108]. The emergence of drug-resistant strains has raised serious concerns about public health regarding these bacterial pathogens [23-100-108]. Despite some reports on the prevalence of food borne bacteria in animal-based foods, livestock, and humans, the extent of these pathogens in animal-based foods within the any region remains insufficiently studied [23-100-108-160]. Furthermore, there is also a concern that the associated risk factors are not well defined, and there is a lack of comprehensive documentation on human infections resulting from food borne exposure [23-100-108]. On the basis of recommendations: a coordinated surveillance and monitoring system for food borne pathogens has been established [23-100-108]. Furthermore, antimicrobial resistance at the national and regional levels across world to develop informed control and prevention strategies against these pathogens [23-100-108-160]. This will generate epidemiological data on risk factors and the incidence of human infections linked to food borne illnesses, focusing on national-level documentation; raise public awareness based on scientific risk analysis of bacterial pathogens responsible for food borne infections [23-100-108]. This will also helped to employ advanced molecular-level characterization techniques, such as whole-genome sequencing, to guide the implementation of improved prevention and control strategies throughout Arab and other countries [23-100-108-160].

Maintenance of hydration and electrolyte balance with oral rehydration is important, especially in patients with vomiting or profuse diarrhea [1-23-100-108]. Travelers with mild non-bloody diarrhea can use loperamide to decrease the frequency of loose stools [23-100-108]. Travelers with moderate illness can consider self-treatment with an antibiotic, and those with bloody diarrhea or severe illness (that keeps them confined to their room) should generally receive antibiotic therapy [23-100-108]. Travelers can use loperamide as an adjunctive therapy to antibiotics taken for moderate or severe travelers' diarrhea [23-100-108]. Azithromycin is preferred for bloody diarrhea or severe illness and is an option for moderate non-bloody diarrhea [23-100-108]. Fluoroquinolones (e.g., ciprofloxacin) can be effective, but resistant strains are increasing in frequency, particularly in Asia [23-100-108]. Other agents are also preferred because fluoroquinolones have been associated with adverse effects, including tendinopathies, QT interval prolongation (a cardiac conduction abnormality), and *Clostridioides difficile* enterocolitis [23-100-108]. If treatment with azithromycin or a fluoroquinolone does not improved the condition within 24 hours, travelers should continue the antibiotic for no longer than 3 days[23-100-108]. A 3-day course of rifaximin is effective for some non-bloody diarrheal illnesses [23-100-108]. Administering certain antimicrobial agents to patients whose clinical syndrome suggests STEC

infection could increase their risk of developing HUS [23-100-108]. Studies of children with STEC O157 infection have shown that early use of intravenous fluids (within the first 4 days of diarrhea onset) might decrease the risk of oligoanuric renal failure [23-100-108].

Antimicrobial-resistant *E. coli* are increasing worldwide [23-100-108]. Carefully weigh the decision to use an antibiotic against the severity of illness; the possibility that the pathogen is resistant; and the risk for adverse reactions (e.g., HUS, rash, other manifestations of allergy), antibiotic-associated colitis, and vaginal yeast infection [23-100-108]. Some studies suggested that loperamide combined with antibiotics can be used safely in many patients [23-100-108]. Due to a potential risk for complications, including toxic megacolon and HUS, avoid treating bloody diarrhea or STEC infection solely with antimotility drugs [23-100-108]. No vaccine is available for *E. coli* infection. Maintenance of hydration and electrolyte balance with oral rehydration is important, especially in patients with vomiting or profuse diarrhea [1-23-100-108]. Travelers with mild non-bloody diarrhea can use loperamide to decrease the frequency of loose stools [23-100-108]. Travelers with moderate illness can consider self-treatment with an antibiotic, and those with bloody diarrhea or severe illness (that keeps them confined to their room) should generally receive antibiotic therapy [23-100-108]. Travelers can use loperamide as an adjunctive therapy to antibiotics taken for moderate or severe travelers' diarrhea [23-100-108]. Azithromycin is preferred for bloody diarrhea or severe illness and is an option for moderate non-bloody diarrhea [23-100-108]. Fluoroquinolones (e.g., ciprofloxacin) can be effective, but resistant strains are increasing in frequency, particularly in Asia [23-100-108]. Other agents are also preferred because fluoroquinolones have been associated with adverse effects, including tendinopathies, QT interval prolongation (a cardiac conduction abnormality), and *Clostridioides difficile* enterocolitis [23-100-108].

Although bismuth subsalicylate and certain antimicrobial agents (e.g., fluoroquinolones, rifaximin) can prevent *E. coli* diarrhea, chemoprophylaxis is not recommended for most travelers [23-100-108-167]. Furthermore, antimicrobial drug use can adversely affect the intestinal microbiota and increase susceptibility to gut infections [23-100-108]. Furthermore, antimicrobial drug use can adversely affect the intestinal microbiota and increase susceptibility to gut infections [23-100-108]. If treatment with azithromycin or a fluoroquinolone does not improved the condition within 24 hours, travelers should continue the antibiotic for no longer than 3 days [23-100-108]. A 3-day course of rifaximin is effective for some non-bloody diarrheal illnesses and consult the physician [23-100-108]. Administering certain antimicrobial agents to patients whose clinical syndrome suggests STEC infection could increase their risk of developing HUS [23-100-108]. Studies of children with STEC: O157 infection have shown that early use of intravenous fluids (within the first 4 days of diarrhea onset) might decrease the risk of oligoanuric renal failure [23-100-108].

1.7. *E. coli* Detection Methods

Escherichia coli is a Gram-negative, facultative, anaerobic bacterium considered to be a commensal organism in the human body [23-100-109-167]. However, the *E. coli* strain O157:H7 is a pathogen that poses a threat to human life by causing several diseases, such as haemolytic–uraemic syndrome (HUS), which may be fatal in some cases [23-100-109-167]. The primary reservoir of *E. coli* O157:H7 is meat, although it has also been isolated from fruits and vegetables [23-100-109-167]. *Escherichia coli* O157:H7 is a food borne pathogen, which causes various health conditions in humans, including fatigue, nausea, bloody diarrhoea and in some cases, even death [23-100-109-167]. In 2017, 15.71% of the total imported food products in Saudi Arabia (SA) were meat-based. In Saudi Arabia (SA), no *E. coli* O157:H7 outbreak has been reported to date, and the prevalence of this pathogen remains unknown [23-100-109-153-167]. However, it has been isolated from several local cattle farms. *Escherichia coli* strains that produce Shiga toxins (Stx1 and Stx2) are called Shigatoxigenic *E. coli* (STEC), while those that produce Shiga-like toxins (verotoxins) are called verotoxigenic *E. coli* (VTEC) [23-100-109-167].

Escherichia coli are gram-negative bacteria that inhabit the gastrointestinal tract [23-100-109-167]. Most types do not cause illness, but 5 pathotypes are associated with diarrhea: enterotoxigenic *E. coli* (ETEC), Shiga toxin–producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC) [23-100-109-167]. In addition, diffusely adherent *E. coli* (DAEC) might also be associated with diarrhea [23-100-109-167]. *E. coli* serotypes are determined by surface antigens (O and H), and specific serotypes tend to cluster within specific pathotypes [23-100-109-167]. Pathotype determination typically is based on testing for virulence genes [23-100-109-167]. Some *E. coli* have virulence genes of >1 pathotype; for example, the O104:H4 strain that caused a 2011 outbreak in Germany produced Shiga toxin and had adherence properties typical of EAEC. STEC also are called verotoxigenic *E. coli* (VTEC), and the term enterohemorrhagic *E. coli* (EHEC) commonly is used to specify STEC strains capable of causing human illness, especially bloody diarrhea and hemolytic uremic syndrome (HUS) [23-100-109-167].

However, at present the conventional method for *E. coli* detection requires several days, especially in cases where *E. coli* concentrations are low [23-100-109-156-167]. Enrichment is a commonly used method for bacterial isolation to

increase the cell counts of target bacteria above other background flora prior to identification [23-100-109-167]. According to FDA-BAM (U.S. Food and Drug Administration-Bacteriological Analytical manual) and other reports, *E. coli* can be enriched with *E. coli* (EC) broth or modified tryptic soy broth (mTSB). However, the enrichment methods are time consuming [23-100-109-167].

Polymerase chain reaction (PCR), using primers against the uidA gene that encodes *β*-D-glucuronidase can be used to identify *E. coli* accurately [1-100-109-167]. PCR detection method has been used to identify a colony on an agar plate, which was formed by plating at least 24-h enriched broth [23-100-109-167]. However, applying PCR detection method directly to the enriched samples has not been evaluated yet. In addition, there is an issue of specificity, since uidA gene is also present in Shigella [23-100-109-156-167]. Commercial molecular tests have increasingly become available and can identify ETEC, EPEC, EAEC, and EIEC through detection of virulence genes [100-109-153-167]. The combination of virulence genes that confer pathogenicity has not been determined for all pathotypes, and *E. coli* sometimes have virulence genes from >1 pathotype due to transfer of mobile genetic elements [23-100-109-167]. Some studies have identified some genes, including the eae gene used to diagnose EPEC, at a similar frequency in stools from healthy people as from those with acute diarrhea [23-100-109-153-167]. Identification of 2 virulence genes in a specimen does not mean they are carried by the same organism. Finally, molecular tests detect genetic material, which does not always correspond to the presence of viable organisms [23-100-109-153-167].

Using PCR or whole-genome sequence analysis to facilitate the recognition of specific *E. coli* pathotypes, state public health and Center for Disease Control and Prevention laboratories can assist in outbreak investigations [100-109-153-167]. When STEC infection is suspected, stool samples should be cultured for *E. coli* O157 and simultaneously tested for Shiga toxins or the genes that encode them [23-100-109-153-167]. Rapid, accurate diagnosis of STEC infection is important because early clinical management decisions can affect patient outcomes, and early detection can help to prevent further transmission [23-100-109-153-167].

1) *E. coli* possesses quite a few virulence factors encoded on mobile genetic elements and/or plasmids or localized in pathogenicity islands [1-155]. These virulence factors are expressed as endotoxins, exotoxins, adhesion, invasion, or iron acquisition factors [155]. Methods for the rapid detection of Escherichia coli and its related toxins are key to minimizing the risk of exposure to food borne pathogens [155]. The experimental study reported by Hariri, 2022 [155] detected *E. coli* in food specimens using culture and polymerase chain reaction (PCR) techniques [155]. One hundred and fifty samples from different types of food, comprising beef (n=60), chicken (n=72), and fish (n=18), were analyzed for the identification of *E. coli* by conventional and PCR methods [155]. One hundred fifty samples from different types of food were purchased from hotels, restaurants, and cafeterias in Mecca city, Saudi Arabia [155]. The results showed that out of 150 food samples, 44 (29.3%) were positive by culture, and 50 (33.3%) were positive by PCR [155]. Significant differences were detected between sample types with culture (p-value < 0.005) [155]. When culture was considered the gold standard, the sensitivity of PCR was 100%, while the specificity was 94.34% [155]. The six-hour pre-enrichment and PCR analysis are reliable in fast detection of *E. coli* in food samples [155]. Hence, the identification of food pathogens using molecular-based methods would become more useful in routine diagnostic laboratories [154-155].

The isolates were grown at 37 °C and 150 rpm for 10 hours [154-155]. Two types of growth media were used: (i) Luria-Bertani (LB) broth for general purposes and (ii) Tryptone Bile X-glucuronide (TBX) agar medium, a selective medium for the enumeration and differentiation of *E. coli* from other coliforms [154-155]. The two growth media were used for the identification of *E. coli* by conventional (culture and biochemical testing) and PCR methods [154-155]. Isolation and identification of *E. coli* were performed by standard microbiological methods. The homogenized samples were transferred into the nutrient broth (5 ml/test tube) and MacConkey agar (Oxoid Limited, Hampshire, United Kingdom) [155]. Any grown colony was picked, Gram stained, subjected to different biochemical tests (sugar fermentation, indole production, methyl-red, Voges-Proskauer, and citrate utilization tests), and then subcultured onto Eosin Methylene Blue (EMB) agar [155].

According to the method reported by Hariri, 2022 [155] the pre-enrichment of samples was performed with some modifications [155]. Approximately 25 g samples were homogenized with 225 mL of buffered peptone water (BPW) medium (Oxoid, CM0509) and then divided into two aliquots [155]. While one aliquot was incubated at 37° C for 24 hours, the other aliquot was subjected to pre-enrichment culture for six hours [155]. The first aliquot was used for DNA extraction by enzyme and freeze/thaw methods, and the second aliquot was used to confirm the presence of *E. coli* by standard culture methods, followed by biochemical and serological confirmatory tests [155]. The DNA was extracted from the first aliquots by the Boil Lysis Method following Ahmed and Dablool [154-155]. Briefly, the aliquot was boiled at 100°C for 10 minutes. Insoluble material was discarded through centrifugation for two minutes, and the supernatant was used as a template in the PCR [155]. A PCR mixture (50 µL) containing one 100 pmol of each primer, 3 µL of DNA

template, and a 25 µL solution of Taq PCR Master Mix polymerase (Promega Corporation, Madison, Wisconsin, United States) was used to conduct the PCR [155]. Using Mastercycler® personal PCR equipment (Eppendorf, Hamburg, Germany), DNA was amplified under the following conditions: heat denaturation at 94 °C for three minutes, followed by 30 amplification cycles (one minute at 94°C, 65 seconds at 62 °C, and 90 seconds at 72 °C), and an elongation step of seven minutes at 72 °C [154]. The primers used were **Afa** FP (5' GCT GGG CAG CAAACT GAT AAC TCT C 3') and **Afa** RP (5' CAT CAA GCT GTT TGTTCG TCC GCC G 3'), which amplified a 480 bp fragment within the conserved **Afa gene sequence** of pathogenic *E. coli* [154-155] using PCR [109-154-155]. The products were electrophoresed in a 1.5% agarose gel for one hour at 120 V, stained with ethidium bromide and visualized under ultraviolet light using a gel documentation system via an ultraviolet transilluminator (UVP BioDoc- It® Imaging System; Ultra-Violet Products Ltd, Cambridge, United Kingdom) [154-155].

E. coli detection in foods is one of the most useful hygienic criteria because of the involvement of the bacterium in causing diseases in addition to its use as an indicator of the presence of other pathogens in foods [109-154-155]. Detection of *E. coli* by culture followed by standard biochemical identification remains the method of choice, especially during outbreaks due to the potential need to compare the results with other typing methods [155]. Therefore, this study reported by Hariri, 2022 [155] confirmed that the PCR achieved was successful in detecting the presence of *E. coli* in food specimens (33.3%) with 100% sensitivity and 94.34% specificity, while the culture method detected *E. coli* in 29.3% of the specimens [155]. The six-hour pre-enrichment of samples and PCR analysis using **Afa gene-specific primers** can reliably and effectively detect *E. coli* rapidly in food samples [109-155]. Hence, the identification of food pathogens using molecular-based methods will become more useful in routine diagnostic laboratories, especially during mass gatherings where the risk of occurrence of food poisoning is high [109- 155]. The importance of this study may be seen in the mass gatherings as the presence of a large number of people in overcrowded conditions considerably increases the risk of occurrence of food poisoning and gastrointestinal diseases caused by uncooked food, so that earlier PCR assays also yielded the simultaneous detection of food-borne pathogens, including *E. coli*, at low concentrations [109-155].

2) Escherichia coli serogroup O157 is the main causative agent of several intestinal and extra-intestinal food borne diseases in humans through the consumption of low-dose contaminated foods such as milk, beef, and vegetables [1-158]. Food borne or waterborne disease occurs when a food borne or waterborne pathogen is ingested with food or water and established itself in the human or animal host [1-159]. It may also occur when a toxigenic food borne or waterborne pathogen establishes itself in a food or water supply and produces different intestinal and extra-intestinal toxins which are ingested [1-159]. The experimental studies regarding the quantitative prevalence of *E. coli* O157 in foods are so limited [159]. Therefore, this study reported by Pakbin et al. (2023) [159] evaluated the quantitative prevalence rate of *E. coli* serogroup O157 in raw milk (n = 144), vegetable salad (n = 174), and minced beef samples (n = 108) using the real-time qPCR SYBR green melting curve method targeting the **rfbA gene** [159]. According to the study conducted by Pakbin et al. (2023) [159] about 2.77%, 10.18%, and 9.19% of raw milk, minced beef, and vegetable salad samples, respectively, were contaminated with *E. coli* O157 [159]. Minced beef and vegetable salad samples were significantly more contaminated than raw milk samples [159]. Population average of *E. coli* O157 in raw milk, minced beef, and vegetable salad samples were 2.22 ± 0.57, 3.30 ± 0.40, and 1.65 ± 0.44 log CFU/ml or gr, respectively [159]. Significantly higher levels of population of *E. coli* O157 were observed in minced beef samples [159]. Minced beef can be regarded as the main food in the transmission of this food borne pathogen [159]. Routine quantitative rapid monitoring is strongly suggested to be carried out to prevent food borne diseases caused by *E. coli* O157 [158]. In this study, *E. coli* O157 (ATCC 43888) and *E. coli* non-O157 (ATCC BAA-2326; ATCC 9637; ATCC 25922; ATCC 27551; ATCC 8739 and ATCC 27325) reference strains were used as controls, respectively [159]. All strains were purchased from Pasteur Institute (Pasteur In.,). Reference strains were grown in tryptic soy broth (TSB, Merck,) and cultured at 37°C for 24 h [159]. Ten-fold dilution series of the reference strains (positive and negative controls) were prepared to design and develop the real-time PCR assay in this study [159]. Serially diluted cultures were subjected to total DNA extraction [159]. Prepared food samples were subjected to total genome extraction by using the SinaClon commercial total DNA extraction kit (SinaClon Co.,) [159]. The quality and quantity of the extracted total genomes were evaluated using the NanoDrop-1000 spectrophotometer (ThermoFisher,) [159]. Concentrations of the all extracted DNA were adjusted to 50 ng. μL^{-1} before real-time PCR assay [158]. DNA templates were kept at −20°C until the further analysis [159]. Real-time PCR was carried out with an Ampliqon 2X SYBR Green Master Mix (Ampliqon,) on a Rotor-Gene Q 6000 real-time PCR machine (Qiagen Corbett,) [159]. Serogroup-specific primers of **rfbA gene**, presenting in *E. coli* O157, used in this study for PCR including forward (5'-CGGACATCCATGTGATATGG-3') and reverse (5'-TTGCCTATGTACAGCTAATCC- 3') primers[158-159]. Real-time PCR tubes contained 10 µl of the 2X real-time PCR master mix, 1 µl of each primer (20 µM), 1 µl of the DNA template (50 ng. µL−1), and sterilized nuclease free water up to a final reaction volume of 20 µl [158-159]. The amplification procedure of real-time PCR was performed using the following conditions: initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 40 s, annealing at 60°C for 40 s, and extension at 72°C for 30 s, followed by a melting step with the increasing temperature from 70 to 95°C with the raising

temperature rate of 0.2°C/s[158-159]. The size of amplicons produced by rfbA primers was 242 bp [159]. The analysis of amplification and melting curves was carried out using the Rotor-Gene 6000 software version 2.3.5 (Qiagen Corbett,) [158-159].

The pathogenic *E. coli* serogroup O157 detection method developed by Pakbin et al. (2023) [159] showed that real-time qPCR SYBR green melting curve analysis is a sensitive and specific assay with a low detection limit for the detection and identification of *E. coli* serogroup O157 in food samples [159]. The limit of detection for rfbA primer set using DNA extracted from serially diluted pure broth culture of *E. coli* serogroup O157 was found to be 10 CFU/ml (1 log of CFU/ml) with the associated Ct value [159]. This study also confirmed that minced beef and vegetable salad samples were significantly more contaminated with E. coil O157 than raw milk samples [158-159]. The population average of *E. coli* serogroup O157 was observed in significantly higher levels in minced beef samples than that in raw milk and vegetable salad samples [158-159]. Sufficient thermal treatments, hygienic practices, and routine quantitative rapid monitoring are strongly recommended to be implemented to prevent food borne diseases and outbreaks caused by *E. coli* O157 through consumption of contaminated foods [158-159].

3) Food hygiene and safety are a major concern in the food industry, and microbiological safety is a particular problem [1-136]. *Escherichia coli* can act as an indicator for the presence of other pathogenic bacteria, and it is detected easily in foods such as pork, beef, and chicken [136]. Thus, *E. coli* detection in foods is one of the most useful hygienic criteria [136]. One of the study reported by Choi et al. 2018 [136] determined the minimum enrichment time for different types of food matrix (pork, beef, and fresh-cut lettuce) in an effort to improve *Escherichia coli* detection efficiency [136]. During this study, fresh pork (20 g), beef (20 g), and fresh cut lettuce (20 g) were inoculated at 1, 2, and 3 Log CFU/g of Escherichia coli [136]. Samples were enriched in filter bags for 3 or 5 h at 44.5 ° C, depending on sample type [135]. *E. coli* cell counts in the samples were enriched in *E. coli* (EC) broth at 3 or 5 h [136]. Furthermore, during this study, one milliliter of the enriched culture medium was used for DNA extraction, and PCR assays were performed using primers specific for **uidA gene** [136]. To detect *E. coli* (uidA) in the samples, a 3–4 Log CFU/mL cell concentration was required [136]. However, *E. coli* was detected at 1 Log CFU/g in fresh pork, beef, and fresh-cut lettuce after 5, 5, and 3-h enrichment respectively [136]. This study also concluded that 5-h enrichment for fresh meats and 3-h enrichment for fresh-cut lettuce in EC broth at 44.5 ° C, and PCR analysis using **uidA** gene-specific primers were found appropriate for the detection of *E. coli* rapidly in food samples [136].

According to Choi et al. 2018 [136], five *E. coli* strains (*E. coli* NCCP11142, *E. coli* NCCP14037, *E. coli* NCCP14038, *E. coli* NCCP14039, and *E. coli* NCCP15661), and Shigella sonnei NCCP14743 strain were cultured in 10 mL tryptic soy broth (TSB, Becton, Dickinson and Company, USA) [136]. One-hundred microliter aliquots were transferred to fresh 10 mL TSB, followed by incubation at 37° C for 24 h [136]. The cultures of the five *E. coli* strains were mixed [136]. Twenty-five milliliters of the *E. coli* mixture and 10 mL S. sonnei were centrifuged at 1,912 g and 4 ° C for 15 min, and the pellets were washed twice with the same volume of phosphate buffered saline (PBS; 0.2 g KH_2PO_4, 1.5 g Na_2HPO_4, 8.0 g NaCl, and 0.2 g KCl in 1 L distilled H_2O [pH 7.4]) [136]. The suspension was diluted with PBS to obtain 3, 4, and 5 Log CFU/mL of inocula, and *E. coli* and S. sonnei were assayed by PCR to determine the detection limit [136]. During this study reported by Choi et al. 2018 [136], ham of pork and round of beef were purchased from a butcher shop, and a fresh-cut lettuce was purchased from a supermarket, located in Seoul, South Korea [136]. Ham of pork and round of beef were cut into 20-g portions with a flames sterilized knife [136]. Fresh pork (20 g, n=4), beef (20 g, n=4), and fresh-cut lettuce (20 g, n=4) were placed aseptically into separate filter bags (3M, St. Paul, MN, USA) [136]. *E. coli* inoculum (0.1 mL) was inoculated onto the surface of the food samples to achieve 1, 2 and 3 Log CFU/g, and samples were massaged 20 times by hand [136]. Samples were then left at room temperature (25 ° C) for 15 min and allowed cell attachment [136]. For the enrichment of the *E. coli*, eighty milliliters of EC broth (BD, USA) were placed into the filter bags, and shaken by hand 30 times [136]. All the samples were incubated at 44.5° C for 0, 4, and 5 h for pork and beef, or 0 and 3, 6, 12 h for fresh-cut lettuce [136]. After enrichment, 1-mL aliquots of the enriched samples were plated onto *E. coli*/coliform petrifilm (3M, USA) to quantify *E. coli* [136]. The plates were incubated at 37 ° C for 24 h, and colonies were manually counted [136].

For DNA extraction, one-milliliter aliquots of inocula and enriched samples were centrifuged at 18,341×g at 4 ° C for 5 min, and supernatants were discarded [136]. Cell pellets were resuspended in 30 µL distilled water and boiled at 100 ° C for 10 min, and the suspensions were centrifuged at 18,341×g and 4 ° C for 3 min [136]. The supernatants were then used for PCR analysis [136]. For PCR analysis, primers targeting the **uidA gene** of *E. coli* (PT-2, GCGAAAACTGTGGAATTGGG PT-3, TGATGCTCCATAACTTCCTG; Size- 252bp) and Shigella identification gene (F255, TCGCATTTCTCTCCCCACCACG ; F413, CCGGATGTGTCTCGGGCAATC: Size bp- 159) were used to differentiate *E. coli* from Shigella [136]. PCR conditions were as follows: 94 ° C for 2 min (initial denaturation), 94 ° C for 20 s (denaturation), 72 ° C for 20 s (extension), and 72 ° C for 2 min (final extension) [136]. Annealing was performed at 53 ° C for uidA or at 62 ° C for the Shigella identification gene for 10 s, and 35 cycles were performed [136]. PCR analysis was performed using

Fast mix French PCR (i-Taq) (iNtRon Biotechnology, Gyeonggi-do, Korea), and PCR products were run on an agarose gel (1.5%) with electrophoresis for 20 min [136]. Target bands were visualized under UV light [136]. In conclusion, this study confirmed that the combination of enrichment and PCR detection method is able to detect *E. coli* via applying PCR with uidA primers to samples directly after 5-h enrichment for fresh meats (pork and beef) and 3-h enrichment for fresh-cut lettuce [136].

4) Escherichia coli O157:H7 is a food borne pathogen, which causes various health conditions in humans, including fatigue, nausea, bloody diarrhoea and in some cases, even death [1-151-167]. According to the Saudi Food and Drug Authority, in 2017, at least 562, 280, and 50 samples of imported beef, chicken and sheep meat, respectively, were tested for the presence of *E. coli* O157:H7 [1-152-167]. Amongst these, *E. coli* O157:H7 was detected respectively 6.80% and 2.20% of the tested beef meat samples [100-152]. During enrichment, samples weighing 25 g selected for enrichment were placed in sterilised sample bags [152]. They were then homogenized with 225 mL of modified tryptone soya broth (mTSB) supplemented with novobiocin to obtain a ratio: mTSB + sample of 1/10 (mass to volume) [152]. The sample bags were massaged by hand and then incubated at 41.5° C for 12–18 h [1-152-167]. After incubation, the samples were subjected to immunomagnetic separation [152]. Subsequently, 50 µL of each sample was streaked out on pre-dried cefixime tellurite sorbitol MacConkey (CT-SMAC) agar plates using sterile loops to obtain many well-isolated colonies and incubated at 37°C for 18–24 h [152]. After incubation, at least five presumptive colonies were selected randomly from each plate and placed into polymerase chain reaction (PCR) tubes containing 10 µL of distilled water (dH_2O) as a preparation step for DNA extraction [152]. The samples were prepared using a PrepMan™ Ultra Sample Preparation Reagent Kit (lot number 1809191) according to the manufacturer's protocol [152]. Real-time PCR (RT-PCR) was performed to amplify the O157:H7-specific target DNA sequences using a MicroSEQ™ *E. coli* O157:H7 Detection Kit (lot number 1804034) according to the manufacturer's protocol [152]. Non-pathogenic *E. coli* ATCC 25922, non-O157 'O111 and O26' and Salmonella strains enteritis and arizona were added as negative controls [152]. Each sample was analysed in triplicate [152].

Contaminated raw meat is the source of 90% of food borne infections [152]. Thirty-one pathogens, including *E. coli* O157:H7, were responsible for 10 million annual episodes of food borne illnesses in the United States [1-152]. According this study reported by Alhadlaq et al., 2023 [152] samples of imported raw meat were obtained from imported meats in the ports of SA, and the prevalence of *E. coli* O157:H7 in these samples was confirmed [152]. Meat products imported from India and Brazil were the most frequently contaminated [152]. The prevalence of *E. coli* O157:H7 was the highest in raw meat products imported from India, posing a threat to public health in the Kingdom of Saudi Arabia [152]. According to Shinde et al. (2020) [76] *E. coli* O157:H7 was frequently isolated from healthy Indian cattle on both organised and non-organised farms in and around the Pune District in India during 2015 [76- 152]. This can be explained by the fact that new generations of cattle may carry the pathogen but may not present any symptoms, thus appearing as healthy livestock [76- 152]. However, the consumption of meat from such asymptomatic carriers of *E. coli* O157:H7 may affect humans, representing a severe public health concern [76-152]. Furthermore, subsequent studies in the same region revealed the presence of *E. coli* O157:H7 isolates resistant to a number of common antibiotics used for livestock animals against this pathogen, including cefotaxime, streptomycin, penicillin G, kanamycin, ampicillin, tetracycline, gentamycin and piperacillin [76-152]. These findings, emphasised the need for the further assessment of imported meat, specifically from India, to ensure public health safety [152]. In another recent study in China, clinical isolates of *E. coli* exhibited high resistance to conventional antibiotics for livestock, including sulfamethoxazole, trimethoprim/ sulfamethoxazole, tetracycline, nalidixic acid and ampicillin [76-152]. This study reported by Alhadlaq et al., 2023 [152] confirmed that the presence of *E. coli* O157:H7 in samples of imported raw meat highlights the need for more regular surveillance at the borders of SA before the products are made available on the market for consumption by the public [152]. These results underscored the necessity of more stringent control protocols for the approval of imported food products, particularly from India and Brazil, which are the major suppliers of meat to SA [76, 152]. Moreover, the detection of *E. coli* O157:H7 isolates should be tested against antibiotics that are commonly used to treat livestock [152]. For the future investigations and as an alternative method, this research work reported by Alhadlaq et al., 2023 [152] suggested tracking different sources of *E. coli* O157 contaminations by clade typing [152].

5) The global burden of food borne illnesses is estimated that 600 million cases with 420, 000 deaths per year [1- 167]. Food-borne illness outbreaks attributed to consumption of raw produce have been reported worldwide with a wide range of causative agents including Salmonella spp., Shigella spp., Escherichia coli (*E. coli*) O157, and Listeria monocytogenes (L. monocytogenes) [1-100- 167]. Fresh fruits and vegetables grown under both conventional and organic agriculture methods can be contaminated with pathogens introduced at different stages of production, such as through soil, organic fertilizer, irrigation water, manure, human handling, and livestock and wildlife [1-100-167]. Raw or partially cooked produce (i.e. sweet basil, spring onion, coriander, cabbage, lettuce, cucumber, and tomato) are generally consumed in Asian countries, including Thailand [1-100-161]. Although consumer interest in organic produce has rapidly grown due to perceptions such as organic farming being more eco-friendly and free from chemicals, organic

agriculture can increase the risk of bacterial contamination due to the use of animal manure-based soil amendments [1-100-161]. However, food-borne pathogen contamination can occur during any of the steps from farm to table [161]. These could compromise microbiological safety and may pose serious public health consequences [161].

In one of the study at Bangkok in Thailand reported by Srisamran et al., (2022) [161], the food samples such as Sweet basil (Ocimum basilicum), spring onion (Alliumcepa var. aggregatum), coriander (Coriandrum sativum), cabbage (Brassica oleracea var. capitata), lettuce (Lactuca sativa), cucumber (Cucumis sativus), and tomato (Lycopersicon esculentum Mill.) were selected based on their popularity and high annual consumption in Thailand [161]. A total of 503 vegetable and fruit samples were collected from May 2018 to February 2019 in Bangkok, Thailand [161]. Six fresh markets and 50 supermarkets were included in this study [161].All samples were purchased and separately packed in a sterile plastic bag [160]. During transportation, the samples were maintained <10 °C on ice in a cool box [161]. All samples were processed within 24 h after collection [161]. Bacterial enumeration method followed the U.S. Food and Drug Administration Bacteriological Analytical manual (U.S. FDA BAM) [138] [1-161].

The conventional culture method was selected based on high sensitivity at low cost with readily available reagents [138-161]. In brief, approximately 25 g of vegetables and 200 g of fruits were added into buffered peptone water (BPW; Difco, Becton Dickinson, Baltimore, MD, USA) to make a triplicate 10-fold serial dilution from 10^{-1} to 10^{-7} [138-161]. One milliliter of each BPW dilution was inoculated in 9-mL lactose broth (Difco) [161]. After overnight incubation at 37 °C, one loopful of suspension was transferred to 9 mL EC broth (Difco) [161]. The tubes were incubated at 45. 5 °C for 24 h [161]. Positive tubes of *E. coli* broth were used to calculate the concentration of fecal coliforms (most probable number (MPN)/g) [161]. One loopful of positive EC broth was streaked onto Levine's eosin–methylene blue (L-EMB) agar (Difco) [161]. The plates were incubated at 37 °C for 24 h [161]. Suspected colonies of *E. coli* were biochemically confirmed using indole and triple sugar iron (TSI; Difco). Typical colonies of *E. coli* on L-EMB agar have a green metallic sheen [161]. The concentrations of *E. coli* (MPN/g) were calculated based on the number of positive EC tubes with confirmed *E. coli* [161]. The detection of *E. coli* O157:H7 was performed according to ISO 16654 with slight modifications [161]. Briefly, raw vegetables (25 g) and fruits (200 g) were added into modified Tryptic soy broth (mTSB) with novobiocin (Merck, Darmstadt, Germany) for selective enrichment of *E. coli* O157:H7[161]. The samples were incubated at 42 °C for 18–24 h and then streaked onto CHROMagar™ O157 (Sharlau, Paris, France) and cefixime–tellurite sorbitol MacConkey (CT-SMAC; Himedia) agar plates [161]. The overall prevalence in these 503 items for fecal coliforms and *E. coli* was 84.3% and 71.4%, with mean concentrations (±standard deviation) of fecal coliforms and *E. coli* being (3.0×105±1.3×106) most probable number (MPN)/g and (1.8×105±1.1×105) MPN/g, respectively [161]. The concentrations of fecal coliforms and *E. coli* were higher in produce sampled from fresh open-air markets than produce from supermarkets. Similarly, these bacterial indicators were higher from produce grown under conventional methods than certified organic produce [161].

This study indicated that fruits and vegetables are important sources of microbial contamination [161]. Hence, monitoring and surveillance of pathogen contamination to produce is needed to strengthen food safety [161]. This study found that the indicator bacteria and Salmonella occur at higher levels in fresh produce grown under conventional than those from certified organic methods [161]. These bacteria occurred at higher levels in produce sampled from fresh open-air markets than produce from supermarkets [161]. Leafy vegetables, including sweet basil, lettuce, and coriander, tended to exhibit higher bacterial contamination, along with produce that was stored overnight in ice boxes than dry refrigeration [161]. Evaluation of microbiological safety of produce from farm to table should be addressed to ensure the quality and safety of fresh produce [1-161].

6) STEC commonly cause gastroenteritis and that results in enterohaemorrhagic gastroenteritis are called (EHEC) [100-162]. The EHEC pathovar has a known pathology by attaching and effacing (A/E) lesions on intestinal epithelial cells, destroying microvilli and inhibiting actin function, and forming pedestals below the site of attachment [1-161, 162]. Infection by EHEC manifests into symptoms such as bloody diarrhea [1-161, 162]. *E. coli* O157:H7 infections are a main cause of hemolytic uremic syndrome, which is associated with severe health implications, including kidney failure, hemolytic anemia, and potential death [161, 162]. Traditional methods for the detection of STEC O157:H7 and other food borne pathogens involved culture media enrichment, isolation on solid selective/differential media, and biochemical identification of isolates, which can take several days to weeks to complete [161, 162]. Over the past few decades, numerous rapid methods have been developed that are typically immunologically (antibody)-based or nucleic acid-based (e.g., PCR) [1-161, 162]. For example, the FSIS method for the detection of STEC O157:H7 and other regulated STEC in meat products involved an initial 15–24 h culture enrichment of a 325 g sample in 975 mL of modified tryptone soy broth (mTSB) [161, 162]. Further screening of the enrichment by PCR, separation of STEC by immunomagnetic separation [161, 162]. This is followed by plating on modified Rainbow Agar, and testing isolated by O-antigen agglutination to acquire a presumptive positive isolate [161, 162]. Presumptive positive isolates are then grown on

sheep blood agar, tested again for O antigen agglutination, identified using the Bruker MALDI Biotyper, and once again screened by PCR [161, 162].

Chen et al. 2022 [162] described a bacteriophage with an integrated luminescence reporter for the detection of *E. coli* O157:H7 [162]. This reporter phage FV10nluc was constructed by cloning the genes for NanoLuc® luciferase [161, 162] into the *E. coli* O157:H7-specific temperate phage FV10 [162]. NanoLuc® produces ATP-independent luminescence after reacting with furimazine substrate [162]. While bacterial luciferase is 77 kDa, NanoLuc® luciferase is a small protein subunit which is only 19 kDa [162]. Its smaller size gives it flexibility in bioreporter construction [162]. The light intensity produced by NanoLuc® has been found to be roughly 150-fold greater than either Firefly or Renilla luciferases [162]. The FV10nluc reporter is added during culture growth, and is capable of detecting as few as five cells of *E. coli* O157:H7 in 40 mL of ground meat slurry within 9 h [162]. This study assessed the potential of using the FV10nluc phage-based luminescence detection assay during enrichment while the sample is in transit [162].

All media components were purchased from Fisher Scientific (Waltham, MA, USA) unless otherwise noted [162]. Lysogeny broth (LB) was prepared by adding 10 g of tryptone powder, 10 g of sodium chloride, and 5 g of yeast per litre of deionized water [162]. All LB media were adjusted to a pH of 7.5 [162]. The LB plates and top agar were made by adding 17 g and 6 g, respectively, of agar to 1 L of the LB media [162]. Further kanamycin (IBI Scientific, Las Vegas, NV, USA) was added to the LB plates at a final concentration of 50 µg/mL [162]. Modified tryptone soya broth (mTSB) was made according to the USDA-FSIS Media and Reagents guideline (USDA-FSIS 2022) [162]. The *E. coli* strain C7927 used in this study by Chen et al., 2022 [162] is a human STEC serotype O157:H7 isolated from an apple cider outbreak [162]. A single colony of *E. coli* O157:H7 C7927 from an LB agar plate was inoculated in 100 mL of LB liquid media and grown overnight at 3^{o} C with shaking (100 rpm) [162]. After overnight incubation, 100 µL of serial dilutions (1:10) were spread onto LB agar plates and enumerated the number of colony-forming units (CFU)/mL of the overnight culture [162].

In order to prepare the bacteriophage, 50 µL of a FV10nluc lysogen of *E. coli* O157:H7 C7927 was inoculated in one liter of LB containing kanamycin (50 µg/mL) and incubated overnight on a shaker at 100 rpm at 37 ^{o} C [162]. Following overnight incubation, 4 mL of chloroform (Fisher Scientific, Waltham, MA, USA) was added to the culture in order to permeabilize the cell membrane and to complete cell lysis [162]. In addition, 48 g of sodium chloride was added and dissolved with constant stirring [162]. The solution was then centrifuged at 15,000 x g for 10 min in order to collect bacteria and cell debris into a pellet. The supernatant containing the phage was collected, and 80 g of polypropylene glycol (Fisher Scientific, Waltham, MA USA) per liter was dissolved into the solution using slow agitation [162]. The solution was stored at 4 ^{o} C overnight to precipitate the phage [162]. The phage solution was then centrifuged at 17,000 x g for 15 min in order to collect the phage [162]. The supernatant was carefully decanted, and the phage pellet was re-suspended in phage buffer (50 mM Tris, 100 mM $MgCl_2$, pH 7.6) and vacuum filtered through a 0.45 µm pore size membrane (Fisher Scientific, Waltham, MA, USA) [162]. Subsequently, the filtrate was passed through a second filter with pore size of 0.1 µm (Fisher Scientific, Waltham, MA, USA). The resulting solution was collected and stored in a sterile 50 mL Falcon screw cap tube at 4 ^{o} C [162]. When using the FV10nluc phage prepared as described above, phage infection assays yielded significant background light upon addition of the Nano-Glo® reagent (Promega Madison WI; see Section 2.4.) [162]. Furthermore, addition of the Nano-Glo® reagent directly to the FV10nluc phage preparations resulted in light emission, suggesting the presence of free luciferase protein in the phage preparations [162].

Thus, a subsequent purification procedure was conducted to remove the free luciferase and reduced the background light emission from the FV10nluc phage preparations, as briefly described by Chen et al., 2022 [162]. An Amicon® Ultra-15 (MilliporeSigma, Burlington, MA, USA) centrifugal filter device that contained a membrane with a 100,000 kDa cutoff was used to separate the luciferase from the FV10nluc phage [162]. Twelve (12) mL of phage preparation was added to each centrifugal filter device, which was then placed in a fixed-angle rotor centrifuge and spun at 5000 x g for 15 min per cycle, for a total of 7 cycles [162]. Since the NanoLuc® (19 kDa) protein is smaller in size compared to the phage (>100 kDa), the phage is concentrated on the filter while the protein is washed out through the filter [1-162]. After every cycle the filtrate was measured for NanoLuc® luminescent activity (described below) and the resuspended phage concentration was determined [162]. The purified and concentrated FV10nluc phage was then resuspended in phage buffer for storage at 4 ^{o} C until further analysis [162].

According to the work by Chen et al., 2022 [162] Coomassie Brilliant Blue G-250 dye (Bio-Rad laboratories, CA USA) was added at 1% wt./vol to top agar in order to enhance the contrast of phage plaques, increasing their visibility and making them easier to count [162]. For plaque assays, 200 µL of an overnight culture of *E. coli* O157:H7 C7927 and 100 µL of serial dilutions of the FV10nluc phage solution were added to melted blue top agar, vortexed, and then poured onto LB plates [162]. Plates were incubated in a 37^{o} C incubator for 18 h and plaques were enumerated the next day for phage titer determination [162]. Plaque assays were conducted in triplicate [162]. The Nano-Glo® reagent (Promega, Madison, WI, USA), which produces ATP-independent luminescence upon the oxidation of furimazine by the NanLuc

luciferase, was used to measure presence of NanLuc luciferase according to the instructions from the manufacturer [162]. An aliquot of 20 µL of Nano-Glo® substrate was added to 1 mL of Nano-Glo® buffer [162]. The resulting reagent was vortexed and either used immediately or stored at 4 ° C for later use [162]. Initial experiments were performed in LB to determine the relationship between phage concentrations (102–105 pfu/mL) and time for the detection of *E. coli* O157:H7, ranging from approximately 2 to 2 x 10^5 CFU per assay [162]. Each assay consisted of a total of 40 mL [162]. Ten-fold dilutions of cells from 10^{S5} to 10^{-11} were prepared from an overnight culture for phage assays [162]. One hundred microliters of each dilution were spread on LB plates in triplicate and incubated overnight at 37 ° C to determine the initial number of CFU/mL in the overnight culture [162].

Growth of the wild-type *E. coli* O157:H7 C7927 and the C7927 FV10nluc lysogen was characterized by measuring the OD600 of cultures grown in mTSB [162]. A colony of C7927 FV10nluc lysogen was tested in 1 mL of LB and 10 µL Nano-Glo® reagent for luminescence prior to inoculation of an overnight growth [162]. A 20 µL aliquot of an overnight culture of wild-type and FV10nluc lysogen strains was inoculated in 100 mL mTSB flasks, then incubated at 37 ° C with shaking at 100 rpm [162]. Growth curves were performed in triplicate. Optical density measurements (OD600) were taken using a BioPhotometer (Eppendorf North America, Enfield, CT, USA) at inoculation and every 25 min for 8 h [162]. In order to evaluate the ability of the purified FV10nluc phage to detect *E. coli* O157:H7 cells in raw ground beef, matrices assays were performed following standard and modified FSIS protocols [162]. Approximately 1.73 x 10^3 pfu/mL final phage concentrations were used with ground beef in stomacher bags and Nalgene bottles, with a final volume of 1 L [162]. This concentration was achieved by the addition of 100 µL of 1.73 x 10^7 pfu/mL phage per assay [161]. All raw ground beef used in this study was purchased at a local grocery store and consisted of 83% lean meat, 17% fat [162]. All ground beef was used immediately after purchase [162].

During the detection of *E. coli*, samples of 325 g of ground beef were put in 975 mL of mTSB broth (1:3 sample to enrichment media) inside a sterile, plain, clear polypropylene stomacher bag without a filter mesh [162]. *E. coli* O157:H7, ranging from approximately 3 to 3 x 104 CFU per bag, were inoculated into each ground beef slurry [162]. Ground beef with mTSB broth and wild-type FV10 phage was used as the negative control [162]. Each sample was hand-massaged for 30 s at the initial time of inoculation [162]. Each assay was performed in triplicate. Luminescence measurements of 1 mL samples were taken after the addition of 10 µL of previously prepared Nano-Glo® reagent using a Sirius luminometer (Berthold Detection Systems, Bad Wildbad, Germany) once per hour [162]. In order to determine the efficacy of the phage-based detection during shipping of ground beef samples, different ratios of beef sample to mTSB were evaluated in selected sample containers [162]. Initial experimentation included 325 g of raw ground beef added to 650 mL of mTSB for a 1:2 sample to media ratio [162]. The same amount of raw ground beef was used with 325 mL of mTSB for a separate experiment at a 1:1 ratio. Each sample was inverted by hand for 30 s at the initial time of inoculation [162]. All Nalgene bottles used were sterile. *E. coli* O157:H7 cell inocula were approximately 3 to 3 x 10^2 CFU per bottle in the 1:2 sample to media ratio and approximately 2 to 2 x 10^2 CFU per bottle in the 1:1 sample to media ratio [162].

This work by Chen et al., 2022 [162] has confirmed FV10nluc specificity to *E. coli* O157:H7 isolates [162]. This research showed that FV10nluc phage can detect roughly five *E. coli* O157:H7 cells in 40 mL raw ground beef slurry after approximately 9 h of enrichment/incubation [162]. Such performance indicated the potential for exploiting this phage infection system as a detection platform coincident with ground beef regulatory test sample shipment [162]. Therefore, this study evaluated the FSIS-recommended sample to media volume of a 325 g raw ground beef sample in 975 mL of mTSB enrichment media, and showed that FV10nluc was able to detect *E. coli* O157:H7, showing higher cell concentrations correlated with high luminescence in real-life application sample sizes [162]. According to Chen et al., 2022 [162], growth at 37° C will be necessary to maximize the growth of STEC and, at least partially, to limit the growth of some of the food borne microbiota [162]. Potentially, a simple and inexpensive heat source, akin to a dry chemical or gel pack hand warmer or insulated boxes, may be assessed for maintaining the required temperature of samples during shipment [162].

7) Diarrheal diseases, primarily due to contaminated food and water, continued to be the world's second leading cause of death in children under five years old, causing 8% of all global deaths, with 80% of the fatalities occurring in South Asia and sub-Saharan Africa [1-163]. One of the most significant microbial risks is associated with the ingestion of water contaminated with feces from humans or animals [1-163]. The quantity of the fecal indicator is essential as the risk of acquiring a waterborne infection increases with the level of contamination [1-163]. The most used methods for detecting coliforms in water samples are membrane filtration, multiple tube fermentation using the Most Probable Number (MPN) method, and presence/ absence tests such as the hydrogen sulfide test [1-163]. However, these methods can achieve a sensitivity of 1 CFU/100 mL between $0.5–7.5/test, they are often laborious, required specialized staff training, are difficult to use in the field, and required long incubation times, typically overnight [1-163]. No test can rapidly detect low levels of viable *E. coli* in water in less than 8 h [163]. Accordingly, UNICEF released a Target Product

Profile (TPP) and guided industries to develop products that accurately determine fecal contamination as quickly as possible [1-163].

A new generation of assays uses engineered bacteriophages to detect pathogens [163]. **Phages are viruses** that infect and multiply within bacteria and are among the most common and diverse entities in the biosphere [1-163]. They exhibit specific host ranges due to complex interactions between the phage attachment proteins and the bacterial cell surface [163]. Phages can be genetically modified to carry the genetic information of a reporter protein that is expressed inside the bacteria during phage replication [1-163]. The use of reporter phages as detection methods has been demonstrated previously to detect pathogens, including Listeria, Mycobacterium, Salmonella, and *E. coli* [163]. Commonly used reporters include, among others, alkaline phosphatase, green fluorescent protein (GFP), bacterial luciferase (lux systems), and NanoLucR luciferase NanoLuc luciferase produces a luminescence signal 100 times stronger than other luciferases and is a smaller protein (19 kDa) that relies on the substrate furimazine to produce a stable, glow-type luminescence [1-163].

Alonzo et al., (2022) [163] used a cocktail of NanoLuc luciferase reporter phages to specifically detect *E. coli* contamination in water [163]. The phage cocktail has a broad *E. coli* host range that allows using the assay on any water source [163]. This assay is performed in a microfluidic chip designed to integrate with an automated instrument prototype (a filtration unit, a liquid handling unit, and a detection unit)for use in the field [163]. The overall cost of the disposable microfluidic device is less than $1, and the associated instrumentation meets the cost targets set forth by the UNICEF TPP, making this platform a suitable alternative to current *E. coli* field test kits [163]. An overnight culture of ATCC 25922 was serially diluted in PBS to yield solutions containing between 1 and 1000 *E. coli* bacteria/mL, as measured by the Most Probable Number (MPN) technique using a Quanti-Tray/2000 system, in triplicate [163]. Each dilution (40 µL) was added in eight replicate wells of a 96-well PVDF filter plate (Corning) and filtered to collect the bacteria [162]. TSB (90 µL) was then added to each well, and the plate was incubated (37 °C, 2 h) [163]. The phage cocktail solution (10 µL) was added to appropriate wells, and the plate was incubated for 1, 1.5, 2, or 2.5 h [163]. After incubation, the solution was filtered through, and the NanoLuc reporter was collected [163]. The filtrate solution was added to a 384-well cellulose filter white plate and again filtered to collect and concentrate the reporter on the nitrocellulose membrane [163]. Nano-Glo substrate (10 µL) was added to each well, and luminescence was measured [163]. These strategies can also be utilized to design specific bacteriophages aimed at detecting other pathogens and indicators in other liquid samples such as urine or beverages [163]. Therefore, this assay, combined with the microfluidic chip platform, Alonzo et al., (2022) [163] proposed a complete automated solution for the detection and semi-quantify *E. coli* at less than 10 MPN/100 mL in 5.5 h by minimally trained personnel [163].

8) According to the study reported by Ranjbar et al., (2017) [164], the insecure food containing pathogenic bacteria, viruses, parasites or harmful chemical elements, causes more than two-hundred diseases and disorders ranging from diarrhea to cancers [1-164]. Shiga toxigenic *Escherichia coli* is one of the common cause of food poisoning in hospitals [1-164]. This research study confirmed that high presence of O157 serogroups, EHEC strains and animal-based antibiotics in cooked foods showed insufficiency of cooking time and temperature in the kitchens of hospitals [164]. According to the study reported by Ranjbar et al., (2017) [164], judicious prescription of antibiotics and attentions to the principles of food safety can reduce the risk of resistant and virulent strains of STEC in hospital foods [164]. Samples of food were collected from the various hospitals in Isfahan province, Iran [164]. All food samples showed normal physical characters including odor, color and consolidation [164]. Totally, 10-g of crushed food samples were homogenized for 2 min in 90 ml of Peptone Water (PW, Merck, Germany) [164]. Then the samples were cultured on 5% sheep blood and MacConkey agar (Merck, Germany) and incubated for 18 to 24 h at 37 °C [164]. Colonies with the typical color and appearance of *E. coli* were picked and streaked again on blood agar plates and re-streaked on EMB agar (Merck, Germany) [164]. All the plates were further incubated for 24 h at 37 °C [164]. The **green metallic sheen** colonies were considered as *E. coli* [164]. The presumptive colonies were biochemically tested for growth on triple sugar iron agar (TSI) and lysine iron agar (LIA), oxidative/fermentative degradation of glucose, citrate utilization, urease production, indol fermentation, tryptophan degradation, glucose degradation (methyl red test) and motility [163]. The colonies were further confirmed using the 16S rRNA-based Polymerase Chain Reaction (PCR) [164]. Bacterial strains were sub cultured overnight in Luria-Bertani broth (Merck, Germany) and further incubated for 48 h at 37 °C [163].

Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Fermentas, Germany) according to manufacturer's instruction [164]. The 10 ml bacterial DNA extract and controls were amplified with 0.5 mM primers (Forward: 5'- F: CGGACATCCATGTGATATGG- 3' and Reverse: 5'- R: TTGCCTATGTACAGCTAATCC- 3') **Target gene 0157**(259 bp), 200 mM of each dNTP (Fermentas, Germany), 2 mM $MgCl_2$, 10 mM KCl PCR buffer and 1.0 U Taq polymerase (Fermentas, Germany) [164]. The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device using the following protocol: 94

°C for 5 min, 40 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and final 72 °C for 5 min[164]. In conclusions, study reported by Ranjbar et al., (2017) [164] identified a large number of Oserogroups, virulence factors, antibiotic resistance genes and pattern of antibiotic resistance in STEC strains recovered from hospital foods [164]. Raw meat, raw chicken and cooked meat, summer season, stx1 and eaeA virulence factors, AEEC subtype, O26 and O157 serogroups, aac (3)-IV, CITM, tetA, dfrA1) and sul1 antibiotic resistance genes, resistance against ampicillin, gentamycin, tetracycline, ciprofloxacin and amikacin and presence of multi-drug resistant strains were the most commonly detected characters in the STEC strains of hospital foods [164]. Presence of O157 serogroups, EHEC strains, animal-based antibiotics and even antibiotic resistance genes which encode resistance against animal-based antibiotics in cooked foods showed insufficiency of cooking time and temperature in the kitchens of hospitals [164]. Due to the low levels of STEC resistance against imipenem, meropenem, streptomycin and cefotaxim antibiotics, occurrence of food poisonings due to the STEC strains in tested Iranian hospitals can be treated with their regular prescription [164].

9) An ideal detection method needs to satisfy five premier requirements – high specificity (detecting only the bacterium of interest), high sensitivity (capable of detecting as low as a single live bacterial cell), short time-to-results (minutes to hours), great operational simplicity (no need for lengthy sampling procedures and use of specialized equipment) and cost effectiveness [1- 164-167]. Culture-based methods are more successful when the growth requirements of target microorganisms are known with culture media being used to enrich, selectively isolate, or discriminate between target microorganisms and other groups [167]. In addition, cultures can be subjected to tests such as colony characteristics, Gram staining reaction, biochemical characterization, and MALDI TOF MS and PCR-sequencing for identification purposes [164, 167]. However, culturing microorganisms is also a slow and laborious process requiring a series of steps and may required the use of adjunct methods (e.g. biochemical, serological, nucleic acid-based methods for conclusive identification) and can take up to a week for bacteria or longer for fungi [1-164, 167]. They also may not be suitable for the rapid detection of microorganisms for on-the-spot or real-time food borne pathogen detection [1-164, 167]. These bacterial groups can be cultured using the appropriate culture media except when they are in the VBNC phase [164, 167]. Therefore, the use of cultural media alone is unreliable. Moreover, they are laborious and have limited sensitivity as they can only be used to detect a subset (culturable bacteria) with a significant population of bacteria not being culturable [164, 167]. The culture-based approaches coupled with other methods such as PCR, Immunoassays, NGS, Biosensors, and **MALDI TOF MS** are increasingly being used for the detection and identification of food borne pathogens [1-164, 167]. In recent times MALDI TOF MS has been successfully used to identify food borne pathogenic bacteria, especially those belonging to the key food borne bacterial pathogenic groups [1-164, 167]. It involves the generation of the peptide mass fingerprint (PMF) of isolated bacterial cultures (and comparing that with the PMF in a selected database for identity typing) [1-164, 167]. For example, culture takes long time to give the results [100-164-167]. On the other hand, PCR, antibody based techniques and biosensors offer shorter waiting time, but these required the use of expensive reagents and sophisticated equipment which make the method expensive[1- 164, 167].

Culture based methods have been the oldest methods in detecting the microorganisms, even the pathogenic strains [100-164-167]. One of the best known examples which showed high success rate and also showed that the method is highly cost-effective is the culture of *E. coli* O157:H7 on Sorbitol MacConkey agar (SMAC) which is based on the principle of fermentation of sorbitol [1-164, 167]. The drawbacks of the SMAC agar can be overcome by the use of chromogenic medium for STEC isolation which has increased specificity and sensitivity [100- 164-167]. Cefsulodin-Irgasan-Novobiocin (CIN) agar, a selective medium known for better discrimination between bacterial species, was used to differentiate *Yersinia enterocolitica* and non *Y. enterocolitica* [164, 167]. Many microorganisms tend to enter starvation mode of metabolism under stress conditions [1- 164-167]. However, they will remain viable but non culturable (VBNC) which cannot be grown on conventional culture (CC) media, but can signal virulent pathways [100- 164-167]. Detection of these pathogens is a major challenge for food safety. Since no colonies will be formed, and other methods such as fluorescent dyes are used for the detection of VBNC bacteria where different dyes are used [1- 164]. Binding of acridine orange to the VBNC pathogens depends on the ratio of DNA to protein in the cells [164]. Actively reproducing cells appear green whereas slow-growing or non-reproducing cells appear orange. Another dye that is used to detect VBNC is fluorescein isothiocyanate, the principle of which is to detect the enzyme activity of living cells [1- 164]. If there is the presence of any living cells, violet or blue color is seen. Bacteriophage-derived high-affinity binding molecules (cell wall binding domains, CBDs) have been recently introduced as tools for the detection and differentiation of Listeria in foods as conventional culture (CC) methods are hampered by lengthy enrichment and incubation steps[1- 164].

Immunoassays include serotyping, immunofluorescence, use of lateral flow devices (LFD) and enzyme-linked immune sorbent assay (ELISA) approaches, with ELISA, being a very accurate immunological method for detecting food borne pathogens and their toxins [1-164, 167]. Immunoassays were developed as these were easier to perform, gave faster result and were less expensive. Hence, generally before directly going into polymerase chain reaction (PCR) based methods, immunoassays are performed [1-164-167]. Enzyme linked immunosorbent assay (ELISA) is one of the most used immunoassays to date. The use of different substrates in ELISA has a major advantage as the substrates will bind

to the respective conjugates specifically and will develop coloration which can be read in an ELISA reader in terms of wavelength [1-164]. The color change is visible to the naked eye [1-164]. However, one of the disadvantages is that the binding of the chemical and conjugate is very specific, and contamination in the intermediate stages can lead to false positive result [1-164]. Monoclonal antibodies are preferred over polyclonal antibody as these have monovalency [100-164-167]. In monoclonal antibodies, the antibody is produced against one specific antigen. While sensitivity and specificity are its major positive features, production is a laborious process and is not cost-effective. Various such experiments have been conducted to detect *L. monocytogenes*, *S. Typhimurium*, L. innocua and *E. coli* [50- 164-167]. In general, the major advantages of PCR are that the process is rapid and sensitive [164]. It is faster than the culture based methods and immunoassays [1-164]. One of the initial advances in molecular cloning and recombinant DNA technology that revolutionized the detection of food borne pathogen is the development of a PCR-based technique [1-164]. SYBR Green is a cyanine dye which immediately binds to all double-stranded DNA present in the sample. During PCR, DNA polymerase amplifies the target sequence which creates the PCR products [1-164]. SYBR Green dye then binds to each new copy of double-stranded DNA. Conventional pathogen detection methods, such as microbiological and biochemical identification, are time-consuming and laborious while immunological or nucleic acid-based techniques required extensive sample preparation and are not amenable to miniaturization for on-site detection [1-164]. Nucleic acid-based methods such as Real-Time PCR and vPCR combined with sequencing approaches are more widely used than immunoassay and NGS-based approaches for pathogen detection [1-164, 167].

Bacteriophages are one such unique biological entity that showed excellent host selectivity and have been actively used as recognition probes for pathogen detection [100- 164]. Biosensors are the devices for pathogen detection that generally consist of three elements, which are a biological capture molecule (probes and antibodies), a method for converting capture molecule target interactions into a signal and an output data [1-164]. The major advantage of the biosensors is that these can detect the pathogens at low detection limits with high specificity and sensitivity, but the biosensors will be required highly specific and expensive instruments, with compatible computer software, to give accurate results [164]. Hence, these methods may not be always cost-effective [1-164]. **Nanobiotechnology** is the latest approach for detection of pathogens. Aptamers are attracting an increasing amount of interest in the development of sensors for proteins, DNA and small molecules [1- 164]. DNA microarray is gaining importance currently and has become a useful tool due to its rapidness, sensitivity and specificity and it allows high throughput analysis [1-164]. Ultrafiltration, immunomagnetic assays (IMS), immunochromatic assay (ICA), flow cytometry (FC) and lyophilization are some of the conventional methods [1-164]. Ultrafiltration has been recognized as an effective procedure for concentration and recovering microbes from large volumes of water and treated waste water [1-164-167].

10) The food poisoning cases investigated during 2003–2005 in Hyderabad are an infective and intoxicating type of food borne diseases caused by Salmonella spp. and *Staphylococcus aureus* [165]. It is interesting that most of the food poisoning cases investigated are caused due to the organism *Staphylococcus aureus* [165]. To prevent food poisoning outbreaks appropriate educative and preventive measures should be taken up [165]. The health authorities should strengthen considerably the food borne disease surveillance system and follow it with efficient education and extension activity or various aspects to conduct appropriate surveillance programmes, so that the real burden of food borne disease can be determined at various national levels [165]. Food borne diseases are one of the health hazards and causes of morbidity and mortality in developing countries [165]. In India there are no systematic studies to understand the types of foods involved and the etiological agent causing the disease. Therefore, a pilot study was proposed to investigate the food poisoning cases, undertaken by the Ronald Ross Institute of Tropical Diseases, which is a referral hospital for food borne diseases in Hyderabad [165]. Food and stool/rectal swabs of the patients affected were collected for microbiological examination[165]. Odds ratio and 95% confidence interval were used to express the statistical significance of the differences [165]. Epidemiological, environmental, and laboratory components indicated that *Staphylococcus aureus* was the etiological agent in most of the cases and in one case Salmonella spp. were the main cause of food poisoning [165]. This study indicated the need to take up food borne disease surveillance under the Indian context and to identify the common high-risk food commodities for microbial contamination and identification [165].

11) Water is considered as a carrier of fecal-borne disease [1-166]. The consumption of such contaminated water can lead to infection with many bacterial, viral and protozoal diseases [1-166]. Bacteria, viruses and protozoa are microbes that may be present in water as pollutants [1-166]. Coliform bacteria are microbes that emerge from the intestinal tracts of warm-blooded animals and exist in soil and vegetation [1-166]. *E. coli* belongs to the *Enterobacteriaceae* family and is described as a facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacterium that contains the enzyme *β*-glucuronidase [1-166]. *E. coli* is commonly found in faeces, and its presence in pharmaceutical preparations is considered as a direct indicator of faecal contamination in those products [1-166]. According to the study of the detection of *E. coli* conducted by Abu-Sini et al., (2023) [166], the sampled water was tested for microbial contamination using the total plate count (TPC) and total coliform count (TCC) methods [166].

Four different types of media were used for the propagation, isolation and detection of *Enterobacteriaceae* [166]. These media included: plate count agar medium (Biolab, Budapest, Hungary), for enumeration of total microorganisms in water samples, m-Endo Agar Les medium (Liofilchem, Italy) for the detection and enumeration of coliforms in water samples, and MacConkey agar and Eosin Methylene Blue medium (Biolab, Budapest, Hungary) for the isolation, enumeration and differentiation of *E. coli* [1-166]. Sterility of the four prepared media types was achieved by autoclaving at 121° C for 15 min [1-166]. The recommended techniques used to determine the faecal contamination of water by *E. coli* are the multiple tube fermentation method and the membrane filtration method, the latter of which was implemented in this study conducted by Abu-Sini et al., (2023) [166]. For all samples, two volumes of about 100 mL were filtered through 0.45 µm pore-sized nylon membrane filters (Agela Technologies, China) using a sterile filtration unit and vacuum pump [166]. The membranes were aseptically removed using sterile forceps, rotated upside down and placed on plates of plate count agar and m-Endo Agar Les, ensuring that no air bubbles were trapped [166]. The plates were then incubated at 35 ± 0.5° C for 22 to 24 h [166]. Colonies of *E. coli* exhibited a distinctive **pink-to-dark red color** with a metallic green sheen in the EMB agar [166].

Coliform density was reported as the number of colonies per 100 mL of sample [166]. Samples of sterile distilled water were used as negative controls [166]. One strain of *E. coli* (ATCC 8739) was mixed with 100 mL sterile distilled water, filtered as above, and used to produce 20–80 coliform colonies per filter [166]. This sample was used as a positive control to enumerate coliform density according to the following equation [166]:

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of original sample filtered}} \times 100$$

According to the study of the detection of *E. coli* conducted by Abu-Sini et al., (2023) [166], if growth covered the entire filtration area of the membrane, or a portion of it without discrete colonies, the results were then reported as "Confluent Growth With or Without Coliforms [166]. If the total number of colonies (coliforms plus non-coliforms) exceeded 200 per membrane, or the colonies were too indistinct for accurate counting, the results were reported as "Too Numerous to Count (TNTC) [166]. *E. coli* isolates were identified based on colonial morphology and with gram staining [166]. Under light microscopy, *E. coli* cells are typically gram negative and are short and rod-like in appearance [166]. Conventional biochemical tests, which included catalase and oxidase tests, were used for further characterization of the bacteria [166]. Further identification of the isolates was performed using the HB010 Hi *E. coli*™ Identification Kit (HiMedia Laboratories, India), which is a standardized colorimetric identification system that uses eight conventional biochemical tests and four carbohydrate utilization tests [166]. Extraction of DNA from propagated bacterial cells was performed using the G-spin Total DNA Extraction Kit according to manufacturer's instructions (iNtRON Biotechnology Seoul, Korea) [166].

Briefly, a pure single colony of *E. coli* was transferred from a nutrient agar plate (Biolab, Budapest, Hungary) into 5 mL nutrient broth media (Biolab, Budapest, Hungary) and then incubated overnight at 37° C until it obtained an OD_{600} value of 0.8~1.0 on a spectrophotometer [166]. Then, 1–2 mL of the bacterial suspension was transferred to a 2 mL tube and centrifuged for 1 min at 13,000 rpm [166]. Next, 200 µL buffer CL, 20 µL Proteinase K and 5 µL RNase A solution were added to the sample tube and mixed using a vortex [166]. The lysate was then incubated at 56° C for 10–30 min in a water bath [166]. Then, 200 µL of buffer BL was added to the sample tube, mixed thoroughly, and incubated at 70° C for 5 min [166]. Finally, the suspension was centrifuged at 13,000 rpm for 5 min and the supernatant was collected and used as a DNA template for PCR [166]. PCR was performed and confirmed the identification of the propagated isolates by detecting the *β*-galactosidase gene of 243bp using the *E. coli*-specific **lacZ3** primers (F: 5" TTGAAAATGGTCTGCTGCTG 3" R: 5" TATTGGCTTCATCCACCACA 3' [166]. All PCR reactions were done using a MultiGene Conventional PCR machine [166]. The gene was amplified using 5 µL of PCR Master Mix 5 (FIREPol® Master Mix Ready to Load, Solis BioDyne, Estonia) [166]. The volume was made up to 25 µL using nuclease free water [166]. Amplification was done by initial denaturation at 95° C for 3 min, followed by denaturation at 95° C for 30 s; annealing temperature of primers was 58° C for 30 s and extension at 72° C for 1 min [166]. The final extension was conducted at 72° C for 10 min and the total reaction was performed for 37 cycles [166]. The amplified PCR products were analyzed by electrophoresis in 1.5% agarose gel at 100 v for 45 min, stained with ethidium bromide, and finally, visualized with UV transilluminator [166].

For the measurement of the antimicrobial drug susceptibility of all *E. coli* isolates, the Kirby–Bauer disk diffusion method was used according to the Clinical and Laboratory Standards Institute guidelines [166]. Susceptibility patterns of the isolates were determined against doxycycline (DO, 30 mcg), ceftazidime (CAZ, 30 mcg), gentamicin (CN, 10 mcg), ciprofloxacin (CIP5, 5 mcg), azithromycin (AZM, 15 mcg), amoxicillin/clavulanic acid (AMC, 30 mcg), levofloxacin (LVX5, 5 mcg) and tetracycline (TE, 30 mcg) [166]. All standard antibiotic discs were obtained from Oxoid (Basingstoke, Hampshire, UK) [166]. The results of antimicrobial testing were recorded as sensitive, intermediate sensitive and

resistant according to zone diameter interpretative standards [166]. *E. coli* ATCC 8739 strain was included as a positive control [166]. This study provides basic information about the presence of *E. coli* in water used for reconstitution of drugs in Jordanian community pharmacies [166]. The current isolation of multidrug-resistant bacteria in most of the sampled water used for drug compounding is an alarming situation that needs special attention by pharmacists and competent authorities [166]. All of the isolated *E. coli* were sensitive to gentamycin, ciprofloxacin and levofloxacin [166]. The susceptibility of the isolates to ceftazidime, doxycycline, tetracycline, azithromycin and amoxicillin/clavulanic acid were 92%, 61%, 46%, 23% and 15%, respectively [166]. This study confirmed the widespread presence of multidrug-resistant bacteria in water intended for reconstituting drugs in local Jordan pharmacies [166].

12) The emerging interest in Cannabis, and Cannabis products is increasing throughout world [168-196]. However, concerns have arisen about the possible contaminations of hemp with *E. coli*, pesticides, heavy metals, microbial pathogens, and carcinogenic compounds during the cultivation, manufacturing, and packaging processes [1-168-196]. For nearly a century, Cannabis has been stigmatized and criminalized across the globe, but in recent years, there has been a growing interest in Cannabis due to the therapeutic potential of phytocannabinoids [168-196]. For the safety and welfare of all users, both medicinal and recreational, there is a necessity for a standardized set of guidelines for cultivation and testing of Cannabis products [168-196]. While dozens of bacterial species found to be present in Cannabis plants, *E. coli*, Salmonella, and Clostridium are a few common potential human pathogenic species shown to be associated with Cannabis [168-196]. There are also concerns for the contamination of Cannabis food products by potentially harmful bacteria including **Listeria and *E. coli*** [168-196]. Most of the microbial contamination occurs during the improper preparation and storage of cannabis products, wet, drying and storage under wet, humid conditions can lead to fungal infections such as powdery mildew and botrytis, and budworm or mite infestations [168-193]. The carcinogenic load of non-medicinal Cannabis, particularly when consumed via smoking, is significant [168-193]. The pathogenic bacterial strains of contaminated Cannabis products were detected by traditional culture based methods, PCR and immunogenic assays. Immunoassays include serotyping, immunofluorescence, use of lateral flow devices (LFD) and enzyme-linked immune sorbent assay (ELISA) approaches, with ELISA, being a very accurate immunological method for detecting food borne pathogens and their toxins [1-164, 167]. Immunoassays were developed as these were easier to perform, gave faster result and were less expensive [1- 167].

2. Conclusion

Food borne diseases are one of the health hazards and causes of morbidity and mortality in developing countries. Food borne diseases cause morbidity and mortality in the general population and they have emerged as a growing public health and economic problem in many countries. Food is an integral part of all social events. Such events expose masses to risk of food-borne infections as the food is prepared under temporary arrangements. In India there are no systematic studies to understand the types of foods involved and the etiological agent causing the disease. In India, food poisoning is common in settings where meals are prepared for large gatherings such as banquets, Langar, messes, religious occasions, and weddings. In India, majority of outbreaks of food borne disease go unreported, unrecognized or un-investigated and may only be noticed after major health or economic damage has occurred. To prevent food poisoning outbreaks appropriate educative and preventive measures should be taken up. However, food-borne pathogen contamination can occur during any of the steps from farm to table. Food borne illness may be the cause of fresh produce contamination by pathogenic bacteria, viruses, and protozoa. This contamination may originate from manure, soil, sewage, surface water, or wildlife. It may also occur during washing, slicing, soaking, packing, and food preparation. One of the most significant microbial risks is associated with the ingestion of water contaminated with feces from humans or animals. ***E. coli* O157:H7** infections are a main cause of hemolytic uremic syndrome, which is associated with severe health implications, including kidney failure, hemolytic anemia, and potential death. Signs and symptoms of *E. coli* O157:H7 infection usually begin three or four days after exposure to the bacteria. But infected person may become ill as soon as one day after exposure to more than a week later.

The major symptoms include: diarrhea, which may range from mild and watery to severe and bloody. Further severe stomach cramping, pain or tenderness, nausea, vomiting, fever, bleeding, seizures, headache, confusion, kidney failure, and constant fatigue in some people. Shiga toxigenic *Escherichia coli* is one of the common cause of food poisoning in hospitals. *E. coli* is commonly found in faeces, and its presence in pharmaceutical preparations is considered as a direct indicator of faecal contamination in those products. Some people also have been infected with *E. coli* after swimming in pools or lakes contaminated with stool. Maintenance of hydration and electrolyte balance with oral rehydration is important, especially in patients with vomiting or profuse diarrhea. Conventional pathogen detection methods, such as microbiological and biochemical identification, are time-consuming and laborious while immunological or nucleic acid-based techniques required extensive sample preparation and are not amenable to miniaturization for on-site detection.

Nucleic acid-based methods such as Real-Time PCR and vPCR combined with sequencing approaches are more widely used than immunoassay and NGS-based approaches for pathogen detection. Immunoassays include serotyping, immunofluorescence, use of lateral flow devices (LFD) and enzyme-linked immune sorbent assay (ELISA) approaches, with ELISA, being a very accurate immunological method for detecting food borne pathogens and their toxins. A new generation of assays uses engineered bacteriophages to detect pathogens. **Bacteriophages** are one such unique biological entity that showed excellent host selectivity and have been actively used as recognition probes for pathogen detection. The major advantage of the biosensors is that these can detect the pathogens at low detection limits with high specificity and sensitivity, but the biosensors will required highly specific and expensive instruments, with compatible computer software, to give accurate results. Antibiotics are efficient, powerful, and the most commonly used treatment against pathogenic *E. coli* in clinical and animal agriculture. Other ways to treat or prevent *E. coli* contamination, such as probiotics, antimicrobial nanoparticles, and radiation treatment had been reported.

Compliance with ethical standard

Acknowledgments

We would like to thank and acknowledge, Karen Viviana Castaño Coronado, Chief Communications Officer (CCO) and CO-Founder of LAIHA (Latin American Industrial Hemp Association), and CEO- CANNACONS, Bogota, D.C., Capital District, Colombia for thoughtful discussions, critical comments, supporting, promoting, encouraging and appreciating this research work. We also thank all the members of LAIHA for supporting and encouraging this research work.

Disclosure of conflict of interest

No conflict of interest to be disclosed

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