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Review

Potential Contamination Sources on Fresh Produce Associated with Food Safety

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ABSTRACT - The health benefits associated with consumption of fresh produce have been clearly demonstrated and encouraged by international nutrition and health authorities. However, since fresh produce is usually minimally processed, increased consumption of fresh fruits and vegetables has also led to a simultaneous escalation of foodborne illness cases. According to the report by the World Health Organization (WHO), 1 in 10 people suffer from foodborne diseases and 420,000 die every year globally. In comparison to other processed foods, fresh produce can be easily contaminated by various routes at different points in the supply chain from farm to fork. This review is focused on the identification and characterization of possible sources of foodborne illnesses from chemical, biological, and physical hazards and the applicable methodologies to detect potential contaminants. Agro-chemicals (pesticides, fungicides and herbicides), natural toxins (mycotoxins and plant toxins), and heavy metals (mercury and cadmium) are the main sources of chemical hazards, which can be detected by several methods including chromatography and nano-techniques based on nanostructured materials such as noble metal nanoparticles (NMPs), quantum dots (QDs) and magnetic nanoparticles or nanotube. However, the diversity of chemical structures complicates the establishment of one standard method to differentiate the variety of chemical compounds. In addition, fresh fruits and vegetables contain high nutrient contents and moisture, which promote the growth of unwanted microorganisms including bacterial pathogens (*Salmonella*, *E. coli* O157: H7, *Shigella*, *Listeria monocytogenes*, and *Bacillus cereus*) and non-bacterial pathogens (norovirus and parasites). In order to detect specific pathogens in fresh produce, methods based on molecular biology such as PCR and immunology are commonly used. Finally, physical hazards including contamination by glass, metal, and gravel in food can cause serious injuries to customers. In order to decrease physical hazards, vision systems such as X-ray inspection have been adopted to detect physical contaminants in food, while exceptional handling skills by food production employees are required to prevent additional contamination.

Key words : Fresh produce, Biological hazards, Chemical hazards, Physical hazards, Detection

"Food safety" refers to the control of foods in the farm to fork continuum to prevent diverse foodborne diseases derived from chemical, microbiological, and physical hazards. Poor hygiene can lead to unsafe foods through contamination by microbes, resulting in a multitude of human health problems. The US Food and Drug Administration (FDA) reported more than 48 million cases of foodborne illnesses annually in the US associated with the consumption of contaminated foods. According to recent statistics, foodborne-related sicknesses resulted in an estimated 128,000 hospitalizations and 3,000 deaths in the US, annually[1]. According to the World Health Organization (WHO), 1 in 10 people fall ill from consumption of contaminated foods every year, and an estimated 420,000 deaths occur[2]. During the last two decades, fruits and vegetables have been identified as one of the main causes of foodborne outbreaks[3].

Fresh produce includes raw and fresh fruits, vegetables, herbs, fungi, and nuts, which are essential components in the diet[4]. Fruits and vegetables are rich in a variety of nutrients, including vitamins, trace minerals, dietary fiber, and many other classes of biologically active compounds which inhibit or prevent chronic diseases such as heart disease, cancer, diabetes, and obesity[5]. Increased rates of fresh produce consumption reported over the past two decades can be attributed to a few factors. First, consumers are becoming more concerned about health through eating correctly, leading to increased demands for a larger variety of domestic

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and imported products during all seasons throughout the year[6]. Secondly, there have been increased efforts by the government to promote healthy foods. As the consumption of fresh produce has increased, the number of foodborne illnesses outbreaks associated with fruits and vegetables has risen[7]. Because fresh produce is mostly consumed raw or after minimal processing, pathogen contamination constitutes a potential health risk[8]. Fresh produce is easily contaminated by chemical, biological, and physical hazards during the transition from farm to fork phase. A recent report by the Center for Science in the Public Interest (CSPI) indicates that one of the highest numbers of outbreaks can be attributed to the fresh produce industry in the US between 2006 and 2016. Fresh produce-related outbreaks constitute not only one of the greatest numbers of total illnesses, but also the largest average number of illnesses per outbreak[9].

In this review, the chemical, biological, and physical hazards associated with food safety in fresh produce will be described, as well as transmission routes, symptoms of contamination, and detection methods.

Chemical contaminants on fresh produce

Chemical contaminations involve the presence of chemicals in a food matrix where their concentration exceeds safe level. Chemical hazards are one of the main causes for foodborne disease outbreaks[10] derived from a variety of sources from harvest to processing. Contamination of fresh produce can occur from soil, sewage, external surfaces, and live animals[11]. Chemical hazards have been considered one of the most serious consumer concerns because of long-term carcinogenic potential from chemical contaminants[12]. The chemical hazards on fresh produce are classified as agro-chemical, natural toxins, and heavy metals (Table 1[13]). Symptoms caused by chemical contaminants span from mild gastroenteritis to fatal cases of hepatic, renal, and neurological syndromes[14]. In recent years, with industrial development and subsequent environmental pollution, foods contaminated by chemicals have become more serious issues[15]. In Nigeria, 400 to 500 children have died annually due to lead poisoning caused by ingestion of foods contaminated with lead-containing soil and dust[16]. According to the Foodborne Disease Surveillance System of the US and Puerto Rico, 257 chemical hazard outbreaks were reported between 2009 and 2015 including 1,024 illnesses and 5 deaths[17].

Agro-chemical hazards

Pesticides

Agricultural pesticides are commonly utilized in farming operations for fruits and vegetables to increase the viability and quality of fresh produce. However, if pesticides are not degraded, these chemical hazards will penetrate plant tissues and can persist into processed products like juices and jams[18]. Another important aspect of pesticides is their transmission to animals or water sources in different ways. Pesticides in fresh foods may result in a number of different health problems such as kidney damage, congenital disabilities, reproductive problems and cancer. In addition, the accumulations of pesticides in the human body may contribute to metabolic degradation[19]. Moreover, pesticides can produce a variety of transformational products (TPs), which can be much more toxic than the parent compounds[20].

Fungicides

Fungicides are widely used chemical agents that provide protection to crops and seedlings in the field and during the storage of foods. Fungicides are unlikely to cause frequent or severe systemic poisoning since a large number of fungicides have low bioavailability and toxicity in mammals[21]. However, the possibilities of chronic health problems and environmental effects have been identified and recently brought to public concern. Dichloran (nitro derivative), flutriafol (triazole), *O*-phenylphenol (biphenyl), prochloraz (imidazole) and tolclofos methyl (thiophosphate) are representative chemical structures available commercially for different crops which have already been proven to have carcinogenic effects in animal and human[22]. To minimize potential hazards, the European Union Commission and the US Environmental Protection Agency (US EPA) established maximum residue limits (MRLs) in fruits and vegetables to ensure that fungicides are not present at certain levels that may influence health threats to the public[23].

Table 1. Example of chemical hazards in food

Chemical hazard	Subcategory of chemical hazard
Agro-chemicals	Pesticides, fungicides, and herbicides
Natural toxins	Mycotoxins (aflatoxin, ochratoxin A, citrinin, and patulin) and plant toxins (cyanogenic glycosides, alkaloids, trypsin inhibitor, and hydrazine)
Heavy metals	Mercury and cadmium

Herbicides

In order to control weeds in crops and increase the yield and quality of produce, modern agricultural production systems rely on the use of herbicides[24]. In general, most herbicides are soil-applied agro-chemicals and their toxicity to mammals is low. Despite the low toxicity, herbicides which are widely used in agriculture, have found their way into public concern due to the presence of their residues identified in foods[25].

Natural toxins

Mycotoxins

Mycotoxins are secondary metabolites produced by fungi. Despite efforts to control fungal contamination, fungi are ubiquitous in the environment and found in most fresh produce[26]. *Penicillium*, *Fusarium* and *Aspergillus* are representative fungi that produce mycotoxin compounds which have a toxigenic impact on food safety. There are more than 1,000 varieties of mycotoxins already reported as causes of several health issues[27,28] from milder symptoms including diarrhea, abdominal pain, or other gastrointestinal problems to more severe complications like cancer[27]. Among varieties of mycotoxins, most of the toxins occur as aflatoxins, ochratoxin A, and patulin[28]. Mycotoxins can contaminate fresh produce via many different routes from pre-harvest to storage since fungi easily colonize crops and contaminate them during harvest or post-harvest stages[29]. Mycotoxins in foods may be partially degraded by physical and chemical methods, as well as irradiation. For example, 54% of patulin can be removed from vegetables or fruits through washing steps. Furthermore, washing rotten or damaged apples results in a 10-fold decrease of the concentration of patulin[30].

Plant toxins

Plant toxins, or phytotoxins, are secondary metabolic compounds produced from plants that play a role in defense mechanisms against insects and fungi. Plant toxins induce a range of negative health effects in humans, from inhibiting an uptake of specific nutrients to carcinogenic properties[31]. Represented plant toxins include potato glycoalkaloids and toxins produced from herbs, such as pyrrolizidine alkaloids and anisatin in certain varieties of star anise[25]. Pyrrolizidine alkaloids are one of the most common phytotoxins having carcinogenic, hepatotoxic, genotoxic and teratogenic properties[32]. Despite the serious health impact of phytotoxins, regulations are not well established in comparison with other chemical hazards such as mycotoxins or herbicides[25]. Until now, there have been a lack of routine methods for the determination of plant toxins due to over 200,000 varieties of secondary plant metabolites reported[33].

Heavy metals

Certain metals (e.g., iron, zinc, manganese and copper) are required micronutrients to maintain proper health. Despite the health benefits of metals, excessive accumulation in animals may induce serious health problems due to the low biodegradability and concentration through the food chain[34]. Industrial processing, pesticides or chemical fertilizers, mining, and automobile exhaust are the main sources of heavy metals in the environment and these compounds are easily transmitted to fresh produce[35]. Heavy metals can seriously deplete specific nutrients in the body, which can lower the natural immunological defenses, impair psychosocial facilities, and cause intrauterine growth retardation. Heavy metal consumption is also associated with malnutrition, and reports have claimed that heavy metals increase the rates of gastrointestinal diseases and cancer[36]. Among various heavy metals, lead (Pb), cadmium (Cd), and mercury (Hg) are the most probable causes of the heavy metal-related diseases[37].

Detection of chemical hazards on fresh produce

US and European governments attempt to define one standard technique to detect chemical hazards from foods, however establishing one standard method to analyze a

Table 2. Detection methods for chemical hazards on fresh produce

Category	Detection method	Reference
Pesticides	Mass chromatography (MS), tandem-MS, liquid chromatography-mass chromatography(LC-MS), and liquid chromatography-time of flight mass chromatography (LC-TOF-MS).	18, 39
Fungicides	Gas chromatography (GC), GC-capillary electrophoresis(CE), LC, and LC-CE	40-42
Herbicides	GC and GC-CE	43
Mycotoxins	Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), and GC	44
Plant toxins	LC - MS, LC- MS/MS, and TOF-MS	45-47
Heavy metals	Inductively coupled plasma-mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS)· MS· potentiometric methods, X-ray fluorescence spectroscopy (XR-FS), noble metal nanoparticles (NMPs), quantum dots (QDs), and magnetic nanoparticles or nanotube	34, 48-50

variety of chemical contaminants is very difficult since all chemical hazards have diverse structures and characteristics[24,38]. Chemical hazard detection methods vary based on the target compounds (Table 2). Classical chemical contamination detection procedures use solvents to extract target analytes. The solvent extraction method is a reliable test, however it is time-consuming, requires a trained technician and large volumes of solvent which produce a large amount of waste[18,24]. In order to reduce chemical waste and analysis time, a chromatography method has been developed to identify chemical compounds. In the past few years, nanostructured materials such as NMPs, QDs and magnetic nanoparticles or nanotubes have been invented for simple, highly sensitive and selective assessment compared with conventional protocols[34].

Biological contaminants on fresh produce

Fresh produce such as whole or fresh-cut fruits and vegetables are important dietary constituents, as they contain high levels of vitamins and minerals[51]. Due to these health benefits, most fresh vegetables and fruits receive minimal processing and are usually consumed as raw. Pathogens can easily contaminate fresh produce, leading to serious health problems[52]. In spite of considerable protection from microbial contamination by low pH, skins, and waxy coatings on fresh produce, the high levels of nutrients and moisture present in fresh produce can create a suitable environment for pathogens[27,29]. Rupturing plant tissues through peeling or cutting releases nutrients and encourages growth of unwanted microorganisms[52]. Microbial contaminations easily occur during the different stages from farm to consumer including production, harvest, processing, storage, transportation and can be introduced from environmental, animal or human sources[53]. Symptoms of foodborne illness range from mild complications as abdominal pain, diarrhea, fever, headaches, vomiting and muscle aches[11], to severe health issues such as autoimmune complications, bloody diarrhea, enterotoxin poisoning, meningitis, septicemia, hemorrhagic colitis, hemolytic uremic syndrome (HUS) and miscarriage in pregnant women[27]. Foodborne pathogens frequently associated with a consumption of fresh produce include viruses (norovirus), parasites, and bacteria (*Salmonella*, *Escherichia coli* O157:H7, *Shigella*, *Listeria monocytogenes*, *Bacillus, Campylobacter*, and *Clostridium*)[9]. Fig. 1 indicates the number of bacterial outbreaks, illnesses and deaths attributed to fresh produce from 2006 to 2016 according to the National Outbreak Reporting System (NORS) from the CDC web database[9]. There are a diverse number of pathogens in existence, however this review focuses on the most common illnesses caused by the bacterial foodborne pathogens *Salmonella*, *E. coli* O157:H7, *Shigella*, *Listeria* and *Bacillus cereus* as well as norovirus and parasites. The possible routes and sources of contamination in fresh fruits and vegetables are diverse, and the exact mechanisms for introduction of pathogens into fresh produce are still unclear[54]. Despite intensive efforts to prove accurate pathways of contamination by biological hazards, the routes to fresh produce contamination in the wild are varied from livestock and other sources such as surface water, soil, and ground water (Fig. 2)[52].

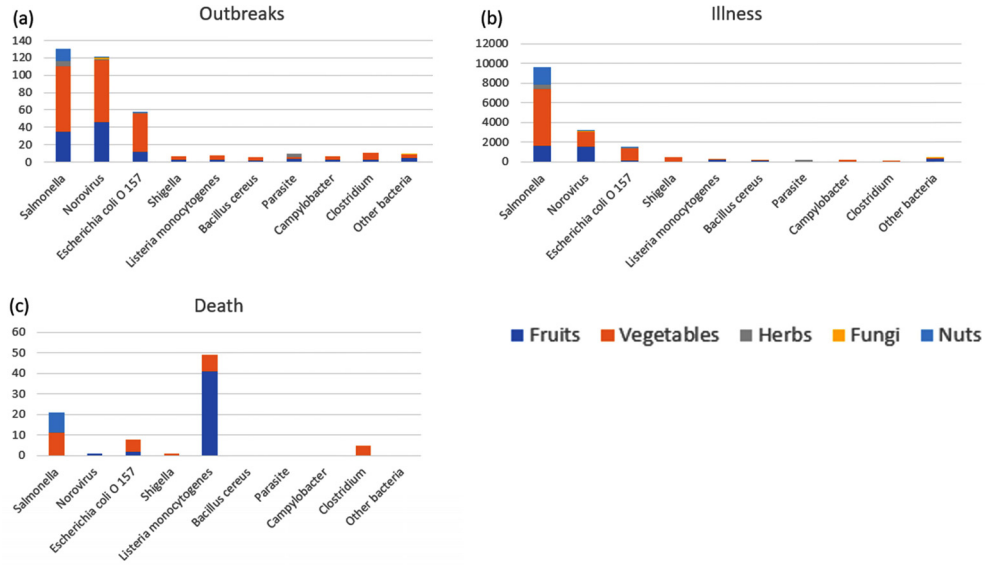

Fig. 1. Incidence of biological foodborne (a) outbreaks, (b) illnesses and (c) death associated with fresh produce reported by the CDC in the US from 2006 to 2016.

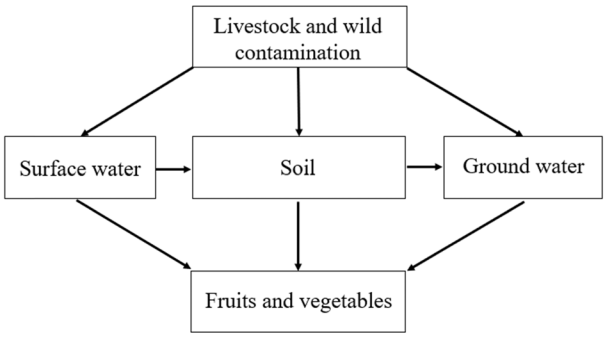

Fig. 2. Environmental risk factors associated with the pre-harvest fresh produce contamination.

Bacterial contaminants on fresh produce

Salmonella

Salmonella is a Gram-negative, non-spore forming bacterium, and is included in the family Enterobacteriaceae. The *Salmonella* genus is composed of two species; *S. enterica* and *S. bongori*[55]. *S. enterica*, which are a main cause of gastroenteritis, is subdivided into hundreds of serovars. *Salmonella* can be easily found in the gastrointestinal tract of animals, from livestock to humans[56]. Previously, most people suspected salmonellosis was attributed to consuming contaminated poultry products, however an increasing number of outbreaks are associated with fresh produce in the US that can be traced back to bacterial contamination by *Salmonella*[57]. In addition, *S. enterica* has been found to easily colonize seeds, leaves, and fruits including watermelon, sprouts, tomatoes, mangoes and lettuce[9,54]. For example, from 2006 to 2016, 131 incidents of foodborne illness outbreaks of *Salmonella* related to vegetables and fruits were reported (Fig. 1)[9]. Approximately 60% of human salmonellosis cases reported by CDC were caused by four serotypes of *Salmonella: S.* Typhimurium, Enteritidis, Newport and Heidelberg[58].

Escherichia coli O157

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobe that commonly inhabits the gastrointestinal tract of mammals and belongs to the family of Enterobacteriaceae[59]. Though most strains of *E. coli* are harmless, several pathogenic strains have been identified that cause serious clinical problems in humans. Enterotoxigenic *Escherichia coli* (ETEC) is a group of pathogens that have the ability to colonize the small intestine in humans, where heat-stable (ST) or plasmid-encoded heat-labile (LT) enterotoxins are produced. Collectively, these organisms cause hundreds of millions of cases of diarrheal diseases each year, particularly in developing countries, with 300,000 to 500,000 estimated deaths of children annually[60].

Six primary pathogenic groups of *E. coli* (enterotoxigenic, enteropathogenic, entero-invasive, enterohemorrhagic, entero-aggregative and adherent-invasive *E. coli*) have been documented. Of these six main pathogen groups, *E. coli* O157 is one of the most common causes of foodborne outbreaks. Pathogenic *E. coli* strains that belong to the Enterohemorrhagic group of *E. coli* (EHEC) are known for verocytotoxin-producing or Shiga-toxin-producing strains[61]. Outbreaks of *E. coli* O157 have been associated with lettuce, unpasteurized apple cider, cantaloupes, and sprouts[9,62]. According to a CDC report, an *E. coli* O157 outbreak linked to romaine lettuce in June 2018 infected 210 people in 36 different states and resulted in 5 deaths[63].

Shigella

Shigella is a Gram-negative, facultative aerobic intracellular pathogen[64]. Species of *Shigella* are most frequently isolated from patients experiencing diarrhea. Between five to fifteen percent of all diarrheal patients worldwide are *Shigella* related[65]. *Shigella* is a member of the Enterobacteriaceae family which closely related to *E. coli*. Though *Shigella* and *E. coli* share very similar DNA sequences, they have remained separate species for clinical reasons[66]. The infective dose for *Shigella* is very low: only 10 cells of *S. dysenteriae* and 500 cells of *S. sonnei* can be infectious[67]. According to CDC reports (Fig. 1), a total of 7 outbreaks, 495 illnesses, and 1 death related to *Shigella* have occurred from 2006 to 2016. In addition, *Shigella* is easily transmissible through person-to-person contact[68].

Listeria monocytogenes

The genus *Listeria* consists of Gram-positive, facultative, non-spore forming bacteria. *Listeria* is represented by seven species: *Listeria monocytogenes*, *innocua*, *welshimeri*, *grayi*, *seeligeri*, *ivanovii* and *marthii.* Among the seven species, *L. monocytogenes* is the primary human pathogen and causes a life-threatening disease known as listeriosis[69]. *L. monocytogenes* represents a serious threat to the food industry because it can survive conventional food processing conditions, such as high salinity, acidity, refrigeration temperatures and low water activity. Though *Listeria* is unable to survive pasteurization temperatures[70], most fresh produce are provided without heat treatment, leading to serious listeriosis outbreaks. In 2015, listeriosis outbreaks occurred in multiple states in the US. All of the patients were over 19, and one of them died[9].

Bacillus cereus

Bacillus cereus is a Gram-positive, spore-forming, motile, aerobic rod that can also grow in anaerobic conditions. *B.*

cereus easily grows within a temperature range of 10 to 50ºC with an optimum between 28 and 35ºC, however members of *Bacillus* survive in a wide variety of environmental conditions due to their ability to form endospores, which are resistant to dehydration, heat, and other physical stresses[71]. *B. cereus* also has the ability to form biofilms on stainless steel[72], demonstrating an increased level of resistance against environmental factors that typically prevent bacterial growth. It also has the ability to contaminate nearly any agricultural product due to its abundance in soil and the ability to form spores and biofilms[73]. *B. cereus* is readily found on a variety of food products, including vegetables, fruits and grains[71].

Non-bacterial contaminants on fresh produce

Norovirus

Foodborne viruses are present in high numbers in human feces. The two types of viruses most frequently implicated in foodborne outbreaks are noroviruses (NoVs) and hepatitis A viruses (HAVs)[74]. Human NoVs are one of the primary sources of viral gastroenteritis around the world, and are the main cause of foodborne illness in Europe[75] and the US[76]. Fresh produce has been identified as a common vehicle for the transmission of foodborne viruses[74]. According to a comprehensive survey of outbreaks identified with fresh produce sources in the US from 2006 to 2016 (Fig. 1), NoVs are the second largest cause of outbreaks (32.8%). Aside from murine strains, NoVs cannot be cultivated *in vitro,* which prevents classification into distinct serotypes and screening from plating[77].

Parasites

Various fruits and vegetables have been identified as vehicles for the transmission of parasites. Parasites associated with vegetable- or fruit-borne outbreaks include helminths such as *Fasciola hepatica*[78], *Ascaris lumbricoides* and *Ascaris suum*[79]. Over 1.5 billion people worldwide have been diagnosed with parasitic infection by at least one species of soil transmitted helminth (STH)[80]. In many developing countries, the use of inappropriate treated wastewater to irrigate vegetables has contributed to contamination with pathogenic parasites. Poor hygienic practices during production by food handlers also contributes to the number of cases of parasitic infeciton[81]. The lack of a globally acceptable methods for the detection and quantification of STH eggs in environmental samples poses a challenge for comparative assessments of egg concentrations in different sample matrices[82].

Symptoms of biological hazards on fresh produce

Biological contaminations of fresh produce induce a variety of symptoms to consumers from mild diarrhea to life-threatening health issues depending on the type of pathogens (Table 3). For instance, the symptoms of salmonellosis, including abdominal cramps, diarrhea, fever, headache, nausea, and vomiting[83], usually develop 8 to 72 h after consumption of contaminated food, and may last from four to seven days. Arthritis-like symptoms may follow three to four weeks after onset of acute symptoms[84]. The typical symptoms of shigellosis are also similar with salmonellosis which include bloody diarrhea, abdominal pain, fever, and malaise[87]. When it comes to *E. coli* O157, the most common etiological problem is hemolytic uremic syndrome (HUS). The virulence level of *E. coli* O157 strains ranges from asymptomatic colonization within the body to potentially lethal HUS disease. Diarrhea-associated HUS is often attributable to Shiga toxin (Stx) produced by pathogenic *E. coli*[85]. Stx-producing *E. coli* (STEC) was named because of the similarity of the toxin generated by the *stx1* gene to

Table 3. Symptoms of biological hazards on fresh produce

Category	Symptoms	Pathogens
Common symptoms	Abdominal cramps, diarrhea, nausea, vomiting, fever, malaise, and headache	All biohazards including bacteriological or non-bacteriological pathogens[78,79,83-91]
Pathogen specific symptoms	Arthritis-like symptoms	*Salmonella*[83, 4]
	Hemolytic uremic syndrome (HUS), and intimin induced life-threatening health complications, especially to infants and the elderly	*E. coli* O157[85, 6]
	Listeriosis which is especially dangerous for elderly and immuno-compromised adults, septicemia or meningoencephalitis, and threat to the unborn child and can lead to miscarriage	*Listeria monocytogenes*[88, 9]
	Anorexia, vertigo and fecal incontinence	Norovirus[91]
	Mild symptoms like eosinophilia to life-threatening health issue depending on the kinds of parasites	Parasites[78, 9]

Table 4. Detection methods for biological hazards on fresh produce

Methods	Assay	Properties	Pathogens
Media/microscopy			
Culturing method	Culturing on selective media	The only culturable pathogen can analyze in selective media, which takes more than 24 h to 48 h.	*Salmonella*, *E. coli* O157, *Shigella*, *Listeria monocytogenes*, and *Bacillus cereus*[68,92,93,95,97]
Microscopic method	Microscopical identification	Separation and concentrations of parasites and quantification through a microscope, but less reliable.	Parasites[82]
Nucleic acid-based			
Polymerase chain reaction (PCR)	Multiplex PCR	Identification more than one species target at a time through amplified specific genes.	All pathogens[68,77,82,92-100]
	Genetic subtyping	Finding differences between unrelated strains	*Salmonella, Shigella*, *Bacillus cereus*, and *Listeria monocytogenes*[68,92,95,97]
	Reverse transcription PCR (RT-PCR)	Reverse transcript from RNA to DNA with reverse transcriptase to read sequence from RNA	*E. coli* O157, and NoVs[94,99]
	Microarray	Thousands of specific DNA sequences to be detected on a small glass or silica slide at the same time.	*Salmonella*, *Shigella*, and *E. coli* O157[68,92,94]
Immunological-based			
Enzyme immunology	Enzyme-linked immunoabsorbent assay (ELISA)	Using natural binding affinity of antibodies to antigens. Antibody combined with an enzyme which can react with a substrate to make fluorescence.	*Salmonella, E. coli* O157, *Listeria monocytogenes*, and NoVs[77,92,94,95]
Non-enzyme immunology	Immunochromatographic	Simple paper-based devices intended to detect the target analytes in a liquid sample (matrix) without the need for specialized and costly equipment	*Salmonella, E. coli* O157, and NoVs[77,92,93]
Biochemical analysis			
Metabolic compounds analyze	Chromatography	Analyze the metabolite compounds through chromatography to identify the pathogen	*Salmonella* and *Listeria monocytogenes*[92,96]
Biosensor			
Biosensor	Biosensor	Recognition signal is generated when specific analytes (immunology or nucleic acid-based parameters) bind to the biological recognition element	*Salmonella, E. coli* O157, and *Shigella*[68,92,93]

the Stx produced by *Shigella dysenteriae*. STEC have an *eae* gene that translates for intimin, which adheres to the outer membrane protein and enables the bacterium to enter the intestinal wall of a host. After the invasion, Stxs which come from STEC can cause life-threatening health complications, especially to infants and the elderly[86]. Additionally, *L. monocytogenes* leads to two types of listeriosis: non-invasive gastrointestinal listeriosis and invasive listeriosis. Both invasive and non-invasive listeriosis are dangerous for elderly and immunocompromised adults, as *Listeria* can manifest as septicemia or meningoencephalitis[88]. Invasive listeriosis is especially dangerous to pregnant women, since perinatal listeriosis is a serious threat to the unborn child and can lead to miscarriage[89]. Additionally, *B. cereus* causes two types of food poisoning that result from different types of toxins, emetic- and entero-toxins, which lead to vomiting and diarrhea, respectively[73]. Emetic toxin syndrome is defined by nausea, vomiting, and abdominal cramping, which occur 1 to 5 h after ingestion of the contaminated food. The illnesses are self-limiting, and recovery usually occurs within 6 to 24 h. Hospitalization is occasionally required due to excessive vomiting, and fatality is rare. The onset of the diarrheal syndrome generally ranges from 8 to 16 h after exposure, and the symptoms resolve themselves in 12 to 14

h[71]. Enterotoxins are proteins causing cytotoxicity marked by fluid accumulation in the ligated ileal loop, dermonecrosis, and lethality in mice[90]. Lastly, the most common acute symptoms of NoVs infection are diarrhea and nausea, followed by vomiting, abdominal pain, fever and fecal incontinence. Various non-specific symptoms are also reported, such as anorexia, thirst and lethargy, headache and vertigo, listed in order of decreasing prevalence. Acute symptoms typically subside after three to four days of illness, whereas nonspecific symptoms can persist for up to 19 days[91].

Detection methods for biological hazards on fresh produce

In order to prevent foodborne illnesses, detection and identification of the specific foodborne pathogen is important and numerous methods have been previously published (Table 4). Conventionally, bacterial pathogens such as *Salmonella*, *E. coli*, *Shigella*, *Listeria monocytogenes* and *B. cereus* can be identified with culture-dependent methods[92,93,97]. Detection of a specific microbial species from a mixed culture using selective media can be accurate however, it usually requires pre-enrichment steps and culturing methods may take more than 1 to 2 days to obtain results[93]. Furthermore, NoVs are impossible to culture on the media[77]. In recent years, molecular microbiological methods such as PCR, rep-PCR and microarray have also been developed. In theory, DNA from a single bacterial cell can be amplified through PCR within 2 h, which is rapid compared to previously described methods[94]. Immuno-based assays have also been created to detect pathogens through specificity of the antigen-antibody reaction, though immunological methods are less sensitive compared to the nucleic acid amplification and cross reactivity with other closely-related species is also a concern[68,98].

With regards to parasites, microscopic methods are traditionally used to identify and quantify eggs of parasites, however newer techniques have been developed. The advent of genomic sequencing and the wealth of data generated by it have markedly increased the feasibility of developing polymerase chain reaction (PCR)-based methods as diagnostic tools for parasites[100]. Another option for detection is analysis of metabolite compounds emitted by pathogen-contaminated food with GC, GC-MS or GT-TOF-MS[96]. Despite the range of detection methods, current protocols are neither fast nor reliable enough to be used in emergency situations[92].

Physical hazards on fresh produce

Physical hazards result from the introduction of unwanted foreign materials into food which cause physical damage to consumers[6]. Physical hazards may involve a wide range of objects, as are listed in Table 5[101]. The primary sources of physical hazards may include the manufacturing environment, raw materials and ingredients, plant equipment, contractors, and employees. In order to detect any contaminants in-line, automated vision systems, X-ray technology, filters and sieves are required[101]. Employee training programs and Good Manufacturing Practices (GMPs) are also included in a physical hazard control program. More effective control programs to prevent physical hazard contamination can be achieved by support from vendors and suppliers, as the magnitude of the potential threat will dictate the appropriate control strategies. A vision system or X-ray inspection may be necessary for the control of glass contamination, while a properly calibrated metal detector may be effective against both ferrous and nonferrous metal contaminants. Human inspection may be required for the detection and removal of dangerous pits and stems[102].

Table 5. Potential physical hazards in the food industry

Material	Injury potential	Sources
Glass	Cuts and bleeding	Bottles, jars, and covers
Wood	Cuts, infection, and choking	Field sources, boxes, and building materials
Stones, gravel	Choking and breaking teeth	Fields and building materials
Insulation	Choking, long-term if asbestos	Building materials
Plastic	Choking, cuts, and infection	Packaging, pallets, and equipment
Personal effects	Choking, cuts, and break teeth	Employees and customers

Summary

Fresh produce is easily contaminated from farm to table by chemical, biological and physical hazards. Among three different type of contaminants, agrochemical, natural toxins and heavy metal contaminants are representative of chemical hazards, which can be detected using solvents, chromatography and nano-techniques. The wide range of chemical structures makes it difficult to establish a single and standardized method to detect target compounds.

Furthermore, fruits and vegetables are a common source of foodborne pathogens, including bacterial pathogens (*E. coli* O157, *Shigella*, *Salmonella*, *Listeria monocytogenes* and *Bacillus cereus*) and non-bacterial pathogens (norovirus and parasites). In order to detect food pathogens and prevent foodborne illnesses, conventional culture dependent methods

have been utilized. However, culturing methods require significant time and effort, and some pathogens, such as noroviruses, are unculturable. To detect foodborne pathogens derived from fresh produce more efficiently, immunological and nucleic acid-based methods should be applied. Finally, physical hazards including glass, plastics and stone contamination of fresh produce result in serious injuries such as choking, broken teeth and bleeding. To avoid serious injuries from physical hazards, vision systems or X-ray inspections are recommended in-line for use by well-trained employees.

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국문요약

신선한 농산물 섭취와 관련된 많은 장점들이 전세계적으로 발표되고 있으며, 지속적인 섭취를 장려하고 있다. 일반적으로 과일과 채소는 최소한으로 가공되기 때문에 천연의 성분들이 건강을 증진시키는 역할을 하기도 하지만 그만큼 질병을 일으킬 수 있는 매개체가 존재할 수 있는 가능성이 매우 높다. 세계 보건기구 (WHO)의 보고서에 따르면 10명 중 1명이 식품에 의해 발생하는 질병으로 고통 받고 있으며, 전 세계적으로 매년 42만 명이 식중독으로 사망하는 것으로 밝혀졌다. 이러한 신선 식품은 농장에서 수확할 때부터 소비자의 식탁에 오르기까지 다양한 경로에서 쉽게 오염 될 수 있다. 본 리뷰논문에서는 신선식품에 의해 발생할 수 있는 질병을 이해하기 위해 화학적, 생물학적, 그리고 물리학적 위험요소로부터 식중독을 일으키는 원인과, 증상, 그리고 검출 방법에 대해서 기술 하였다. 화학적 위험요소의 대표적인 예로는 농약(살충제, 살균제, 및 제초제), 천연 독소 (곰팡이 독소 및 식물 독소), 그리고 중금속 (수은 및 카드뮴) 등이 있으며 이는 크로마토그래피 및 나노 기술 등을 이용하여 검출 할 수 있다. 하지만, 여러 실험에도 불구하고 화학적 위험 요소는 그 구조가 다양하기 때문에 위험 요소를 검출하는 하나의 표준 방법을 수립하기 힘들다. 신선한 과일과 채소는 영양분과 수분이 풍부하기 때문에 박테리아성 병원균 (*Salmonella*, *E. coli* O157: H7, *Shigella*, *Listeria monocytogenes*, *Bacillus cereus*), 바이러스 또는 기생충에 의해 쉽게 오염이 되며, 이를 검출하기 위해 주로 다양한 분자 생물학적 기술이 사용되고 있다. 마지막으로 물리적 위험요소인 유리, 금속, 자갈 등과 같은 매개체는 가공 공정 중에 식품에 유입되어 소비자에게 신체적 상해를 줄 수 있다. 이러한 위험요소를 줄이기 위해서 X-선 검사와 같은 투시 시스템을 이용하여 위해물질을 탐지하거나, 생산에 관여하는 직원 교육을 통해 2차 감염을 줄일수 가 있다.

References

1. US Food and Drug Administration.: Foodborne illness-causing organisms in the US: what you need to know. *The U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition*, 888 (2009).
2. World Health Organization.: Food safety: fact sheet no. 399. World Health Organization, Geneva; https://www.who.int/en/news-room/fact-sheets/detail/food-safety Accessed Oct. 4th, (2018).
3. Westrell T., Ciampa N., Boelaert F., Helwigh B., Korsgaard H., Chriél M., Ammon A., and Mäkelä P.: Zoonotic infections in Europe in 2007: a summary of the EFSA-ECDC annual report. *Eurosurveillance*., **14**, 19100 (2009).
4. Beuchat L.R.: Pathogenic microorganisms associated with fresh produce. *J. Food Prot*., **59**, 204-216 (1996).
5. Alegbeleye O.O., Singleton I., and Sant'Ana A.S.: Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiol.*, **73**, 177-208 (2018).
6. Pollack, S.L.: Consumer demand for fruit and vegetables: the US example. *Changing structure of global food consumption and trade*., U.S. Department of Agriculture, Economic Service, **6**, 49-54 (2001).
7. Warriner K., Huber A., Namvar A., Fan W., and Dunfield K.: Recent advances in the microbial safety of fresh fruits and vegetables. *Adv. Food Nutr. Res*., **57**, 155-208 (2009).
8. Callejón R.M., Rodríguez-Naranjo M.I., Ubeda C., Hornedo-Ortega R., Garcia-Parrilla M.C., and Troncoso A.M.: Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne. Pathog. Dis.,* **12**, 32-38 (2015).
9. CDC (Centers for Disease Control and Prevention)(2018).: *National outbreak reporting system*; https://wwwn.cdc.gov/NorsDashBoard/Default.aspx. Accessed Oct. 4th, (2018).
10. Faille C., Cunault C., Dubois T., and Bénézech T.: Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns. *Innov. Food Sci. Emerg. Technol*., **46**, 65-73 (2017).
11. Marriott N.G., Schilling M.W., and Gravani, R.B.: Food contamination sources. *Principles of Food Sanitation*, Springer, New York, pp. 83-91 (2018).
12. Mastovska, K.: Modern analysis of chemical contaminants in food. *Food Safety Magazine*.; https://www.foodsafetymagazine.com/magazine-archive1/februarymarch-2013/modern-analysis-of-chemical-contaminants-in-food/ Accessed Oct. 4th, (2018).
13. Jackson L.S.: Chemical food safety issues in the United States: past, present, and future. *J. Agric. Food Chem*., **57**, 8161-8170 (2009).

14. Rather I.A., Koh W.Y., Paek W.K., and Lim J.: The sources of chemical contaminants in food and their health implications. *Front. Pharmacol*., **8**, 830 (2017).
15. Song Q., Zheng Y.-J., Xue Y., Sheng W.G., and Zhao M.R.: An evolutionary deep neural network for predicting morbidity of gastrointestinal infections by food contamination. *Neurocomputing*., **226**, 16-22 (2017).
16. Tirima S., Bartrem C., von Lindern I., von Braun M., Lind D., Anka S.M., and Abdullahi A.: Food contamination as a pathway for lead exposure in children during the 2010-2013 lead poisoning epidemic in Zamfara, Nigeria. *J. Environ. Sci*., **67**, 260-272 (2018).
17. Dewey-Mattia D., Manikonda K., Hall A.J., Wise M.E., and Crowe S.J.: Surveillance for foodborne disease outbreaks—United States, 2009-2015. *MMWR. Surveill. Summ*., **67**, 1 (2018).
18. Jin B., Xie L., Guo Y., and Pang G.: Multi-residue detection of pesticides in juice and fruit wine: A review of extraction and detection methods. *Food Res. Int*., **46**, 399-409 (2012).
19. Kher S.V., De Jonge J., Wentholt M.T., Deliza R., de Andrade J.C., Cnossen H.J., Luijckx N.B.L., and Frewer L.J.: Consumer perceptions of risks of chemical and microbiological contaminants associated with food chains: a cross-national study. *Int. J. Consum. Stud*., **37**, 73-83 (2013).
20. Aga D. and Thurman E.: Formation and transport of the sulfonic acid metabolites of alachlor and metolachlor in soil. *Environ. Sci. Technol*. *Lett*., **35**, 2455-2460 (2001).
21. Blasco C., Font G., Mañes J., and Picó Y.: Solid-phase microextraction liquid chromatography/tandem mass spectrometry to determine postharvest fungicides in fruits. *Anal. Chem*., **75**, 3606-3615 (2003).
22. Blasco, C., Pico Y., and Font G.: Monitoring of five postharvest fungicides in fruit and vegetables by matrix solid-phase dispersion and liquid chromatography/mass spectrometry. *J. AOAC. Int.,* **85**, 704-711 (2002).
23. Food Quality Protection Act.: Public Law 104-170. *US Code of Federal Regulations*; https://www.govinfo.gov/app/details/PLAW-104publ170 Accessed Oct. 4th, (2018).
24. Tadeo J., Sanchez-Brunete C., Perez R., and Fernández M.: Analysis of herbicide residues in cereals, fruits and vegetables. *J. Chromatogr. A*., **882**, 175-191 (2000).
25. van Egmond H.P.: Natural toxins: risks, regulations and the analytical situation in Europe. *Anal. Bioanal. Chem.,* **378**, 1152-1160 (2004).
26. Murphy P.A., Hendrich S., Landgren C., and Bryant C.M.: Food mycotoxins: an update. *J. Food Sci.*, **71**, R51-R65 (2006).
27. Yeni F., Yavaş S., Alpas H., and Soyer Y.: Most common foodborne pathogens and mycotoxins on fresh produce: a review of recent outbreaks. *Crit. Rev. Food Sci. Nutr*., **56**, 1532-1544 (2016).
28. van Egmond H.P., Schothorst R.C., and Jonker M.A.: Regulations relating to mycotoxins in food. *Anal. Bioanal. Chem*., **389**, 147-157 (2007).
29. Forsythe S.J.: The microbiology of safe food. *Wiley*, John Wiley & Sons, New York, pp. 26 -29 (2011).
30. Drusch S., and Ragab W.: Mycotoxins in fruits, fruit juices, and dried fruits. *J. Food Prot*., **66**, 1514-1527 (2003).
31. Novak W.K. and Haslberger A.G.: Substantial equivalence of antinutrients and inherent plant toxins in genetically modified novel foods. *Food Chem. Toxicol*., **38**, 473-483 (2000).
32. Wiedenfeld H. and Edgar J.: Toxicity of pyrrolizidine alkaloids to humans and ruminants. *Phytochem. Rev.,* **10**, 137-151 (2011).
33. Hartmann T.: From waste products to ecochemicals: fifty years research of plant secondary metabolism. *Phytochemistry.,* **68**, 2831-2846 (2007).
34. Aragay G., Pons J., and Merkoçi A.: Recent trends in macro-, micro-, and nanomaterial-based tools and strategies for heavy-metal detection. *Chem. Rev.,* **111**, 3433-3458 (2011).
35. Han W.-Y., Zhao F.-J., Shi Y.-Z., Ma L.-F., and Ruan J.-Y.: Scale and causes of lead contamination in Chinese tea. *Environ. Pollut.,* **139**, 125-132 (2006).
36. Valko, M., Morris H., and Cronin M.: Metals, toxicity and oxidative stress. *Curr. Med. Chem.,* **12**, 1161-1208 (2005).
37. Flora S.J.: Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxid. Med. Cell. Longev*., **2**, 191-206 (2009).
38. Turner N.W., Subrahmanyam S., and Piletsky S.A.: Analytical methods for determination of mycotoxins: a review. *Anal. Chim. Acta.*, **632**, 168-180 (2009).
39. LeDoux M.: Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades. *J. Chromatogr. A*., **1218**, 1021-1036 (2011).
40. Tena M., Rios A., Valcarcel M., and Sanchez-Alarcon M.: Supercritical fluid extraction of organophosphorus pesticides from organge samples: Effect of solid additives on recovery. *Chromatographia.,* **46**, 524-528 (1997).
41. Al-Alam J., Bom L., Chbani A., Fajloun Z., and Millet M.: Analysis of dithiocarbamate fungicides in vegetable matrices using HPLC-UV followed by atomic absorption spectrometry. *J. Chromatogr. Sci*., **55**, 429-435 (2017).
42. Konášová R., Dytrtová J.J., and Kašička V.: Determination of acid dissociation constants of triazole fungicides by pressure assisted capillary electrophoresis. *J. Chromatogr. A*., **1408**, 243-249 (2015).
43. Tadeo J., Sanchez-Brunete C., Garcia-Valcarcel A., Martinez L., and Pérez R.: Determination of cereal herbicide residues in environmental samples by gas chromatography. *J. Chromatogr. A*., **754**, 347-365 (1996).
44. Zheng M.Z., Richard J.L., and Binder J.: A review of rapid methods for the analysis of mycotoxins. *Mycopathologia*., **161**, 261-273 (2006).
45. Verpoorte R. and Niessen W.: Liquid chromatography coupled with mass spectrometry in the analysis of alkaloids. *Phytochem. Anal*., **5**, 217-232 (1994).
46. Holstege D.M., Puschner B., and Le T.: Determination of

grayanotoxins in biological samples by LC-MS/MS. *J. Agr. Food. Chem*., **49**, 1648-1651 (2001).
47. Li S.-L., Song J.-Z., Qiao C.-F., Zhou Y., and Xu H.-X.: UPLC-PDA-TOFMS based chemical profiling approach to rapidly evaluate chemical consistency between traditional and dispensing granule decoctions of traditional medicine combinatorial formulae. *J. Pharm. Biomed. Anal*., **52**, 468-478 (2010).
48. Pohl P.: Determination of metal content in honey by atomic absorption and emission spectrometries. *Trends Anal. Chem*., **28**, 117-128 (2009).
49. Mimendia A., Legin A., Merkoçi A., and del Valle M.: Use of sequential injection analysis to construct a potentiometric electronic tongue: Application to the multidetermination of heavy metals. *Sens. Actuator B-Chem*., **146**, 420-426 (2010).
50. Wang L., Ma W., Xu L., Chen W., Zhu Y., Xu C., and Kotov N.A.: Nanoparticle-based environmental sensors. *Mater. Sci. Eng. R Rep*., **70**, 265-274 (2010).
51. Beuchat L.R.: Surface decontamination of fruits and vegetables eaten raw: a review. *World Health Organization.,* 42 (1998).
52. Harris L., Farber J., Beuchat L., Parish M., Suslow T., Garrett E., and Busta F.: Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf*., **2**, 78-141 (2003).
53. Food and Drug Administration.: *Analysis and evaluation of preventive control measures for the control and reduction/ elimination of microbial hazards on fresh and fresh-cut produce*; https://www.fda.gov/Food/FoodScienceResearch/ucm091363.htm. Accessed Oct. 4th, (2018).
54. Sivapalasingam S., Friedman C.R., Cohen L., and Tauxe R.V.: Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot*., **67**, 2342-2353 (2004).
55. Brenner F., Villar R., Angulo F., Tauxe R., and Swaminathan B.: *Salmonella* nomenclature. *J. Clin. Microbiol*., **38**, 2465-2467 (2000).
56. Lan R., Reeves P.R., and Octavia S.: Population structure, origins and evolution of major *Salmonella enterica* clones. *Infect. Genet. Evol.*, **9**, 996-1005 (2009).
57. Tauxe R., Kruse H., Hedberg C., Potter M., Madden J., and Wachsmuth K.: Microbial hazards and emerging issues associated with produce a preliminary report to the national advisory committee on microbiologic criteria for foods. *J. Food Prot*., **60**, 1400-1408 (1997).
58. Dunkley K., Callaway T., Chalova V., McReynolds J., Hume M., Dunkley C., Kubena L., Nisbet D., and Ricke S.: Foodborne *Salmonella* ecology in the avian gastrointestinal tract. *Anaerobe.*, **15**, 26-35 (2009).
59. Garrity G.M., Brenner D.J., Krieg N.R., and Staley J.T.: Part B: The Gammaproteobacteria. *Bergey's Manual of Systematic Bacteriology. Volume 2: The Proteobacteria,* Springer, New York (2005).
60. World Health Organization.: Future directions for research on enterotoxigenic *Escherichia coli* vaccines for developing countries. *Wkly. Epidemiol. Rec.,* **81**, 97-104 (2006).
61. Graeme K.A. and Pollack C.V.: Heavy metal toxicity, part I: arsenic and mercury. *J. Emerg. Med.,* **16**, 45-56 (1998).
62. Steele B., Murphy N., Arbus G., and Rance C.: An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. *J. Pediatr.*, **101**, 963-965 (1982).
63. U.S. Food and Drug Administraction: *Multistate Outbreak of E. coli O157: H7 Infections Linked to Romaine Lettuce*; https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm604254.htm. Accessed Oct. 4th, (2018).
64. Hale T.L.: Genetic basis of virulence in *Shigella* species. *Microbiol. Rev.*, **55**, 206-224 (1991).
65. Kotloff K.L., Winickoff J.P., Ivanoff B., Clemens J.D., Swerdlow D.L., Sansonetti P.J., Adak G., and Levine M.: Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull. World. Health. Organ*., **77**, 651-666 (1999).
66. Portugal F.H., Colwell R.R., Huq A., and Chowdhury A.: Compositions and methods for differentiating among *shigella* species and *shigella* from *E. coli* species, *Google Patents* (2011).
67. Kothary M.H. and Babu U.S..: Infective dose of foodborne pathogens in volunteers: a review. *J. Food Saf.*, **21**, 49-68 (2001).
68. Warren B., Parish M., and Schneider K.: *Shigella* as a foodborne pathogen and current methods for detection in food. *Crit. Rev. Food Sci. Nutr*., **46**, 551-567 (2006).
69. Graves L.M., Swaminathan B., and Hunter S.B.: Subtyping *Listeria monocytogenes. Food science and technology.* Marcel dekker, New York, pp. 283 (2007).
70. Jadhav S., Bhave M., and Palombo E.A.: Methods used for the detection and subtyping of *Listeria monocytogenes. J. Microbiol. Methods.*, **88**, 327-341 (2012).
71. Schoeni J.L. and Lee Wong A.C.: *Bacillus cereus* food poisoning and its toxins. *J. Food Prot*., **68**, 636-648 (2005).
72. Andersson A. and Rönner U.: Adhesion and removal of dormant, heat-activated, and germinated spores of three strains of *Bacillus cereus. Biofouling*., **13**, 51-67 (1998).
73. Kramer J.M. and Gilbert R.J.: *Bacillus cereus* and other *Bacillus* species. *Foodborne bacterial pathogens*. Marcel dekker, New York, pp. 21-70 (1989).
74. Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO).: Microbiological hazards in fresh leafy vegetables and herbs: meeting report. Vol. 14. World Health Organization (2008).
75. Kroneman A., Verhoef L., Harris J., Vennema H., Duizer E., Van Duynhoven Y., Gray J., Iturriza M., Böttiger B., and Falkenhorst G.: Analysis of integrated virological and epidemiological reports of Norovirus outbreaks collected within the Foodborne Viruses in Europe network from 1 July 2001 to 30 June 2006. *J. Clin. Microbiol.*, **46**, 2959-2965 (2008).
76. Scallan E., Hoekstra R.M., Angulo F.J., Tauxe R.V., Widdowson M.-A., Roy S.L., Jones J.L., and Griffin P.M.:

Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis*., **17**, 7 (2011).
77. Vinjé J.: Advances in laboratory methods for detection and typing of norovirus. *J. Clin. Microbiol.*, 373-381(2014).
78. Bjorland J., Bryan R.T., Strauss W., Hillyer G.V., and McAuley J.B.: An outbreak of acute fascioliasis among Aymara Indians in the Bolivian Altiplano. *Clin. Infect. Dis.*, **21**, 1228-1234 (1995).
79. Räisänen S., Ruuskanen L., and Nyman S.: Epidemic ascariasis—evidence of transmission by imported vegetables. *Scand. J. Prim. Health. Care.*, **3**, 189-191 (1985).
80. World Health Organization (WHO).: Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected tropical diseases 2015. Vol. 3. World Health Organization (2015).
81. Gupta S., Satpati S., Nayek S., and Garai D.: Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. *Environ. Monit. Assess.*, **165**, 169-177 (2010).
82. Amoah I.D., Singh G., Stenström T.A., and Reddy P.: Detection and quantification of soil-transmitted helminths in environmental samples: a review of current state-of-the-art and future perspectives. *Acta. Trop.*, **169**, 187-201 (2017).
83. Schneider K.R., Schneider R.G., Hubbard M.A., and Richardson S.: Preventing foodborne illness: Salmonellosis1. *IFAS*, University of Florida (2000).
84. Kendall P.: Bacterial foodborne illness. Food and nutrition series. *Food Nutr. Ser*., **9**, 300 (2003).
85. Tarr P.I., Gordon C.A., and Chandler W.L.: Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *The lancet*., **365**, 1073-1086 (2005).
86. Boyce T.G., Swerdlow D.L., and Griffin P.M.: *Escherichia coli* O157: H7 and the hemolytic-uremic syndrome. *N. Engl. J. Med.*, **333**, 364-368 (1995).
87. Sansonetti P.: Genetic and molecular basis of epithelial cell invasion by *Shigella* species. *Rev. Infect. Dis.*, **13**, S285-S292 (1991).
88. Disson O., Grayo S., Huillet E., Nikitas G., Langa-Vives F., Dussurget O., Ragon M., Le Monnier A., Babinet C., and Cossart P.: Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. *Nature*., **455**, 1114 (2008).
89. Lowe D.E., Robbins J.R., and Bakardjiev A.I.: Animal and human tissue models of vertical *Listeria monocytogenes* transmission and implications for other pregnancy-associated infections. *Infect. Immun.*, 00801-17 (2018).
90. Montville T.J. and Matthews K.R.: Principles which influence microbial growth, survival, and death in foods. *Food microbiology fundamentals and frontiers*. American society for microbiology. Washington, D.C., pp. 13-29 (1997).
91. Goller J., Dimitriadis A., Tan A., Kelly H., and Marshall J.: Long-term features of norovirus gastroenteritis in the elderly. *J. Hosp. Infect.*, **58**, 286-291 (2004).
92. Lee K.-M., Runyon M., Herrman T.J., Phillips R., and Hsieh J.: Review of *Salmonella* detection and identification methods: aspects of rapid emergency response and food safety. *Food Contr*., **47**, 264-276 (2015).
93. Gehring A.G., Brewster J.D., Irwin P.L., Tu S.-I., and Van Houten L.J.: 1-Naphthyl phosphate as an enzymatic substrate for enzyme-linked immunomagnetic electrochemistry. *J. Electroanal. Chem.*, **469**, 27-33 (1999).
94. Deisingh A. and Thompson M.: Strategies for the detection of *Escherichia coli* O157: H7 in foods. *J. Appl. Microbiol.*, **96**, 419-429 (2004).
95. Zunabovic M., Domig K.J., and Kneifel W.: Practical relevance of methodologies for detecting and tracing of *Listeria monocytogenes* in ready-to-eat foods and manufacture environments-A review. *LWT.*, **44**, 351-362 (2011).
96. Spadafora N.D., Paramithiotis S., Drosinos E.H., Cammarisano L., Rogers H.J., and Müller C.T.: Detection of *Listeria monocytogenes* in cut melon fruit using analysis of volatile organic compounds. *Food Microbiol.*, **54**, 52-59 (2016).
97. Collee J., Duguid J., Fraser A., Marmion B., and Simmons A.: Laboratory strategy in the diagnosis of infective syndromes. *Mackie and McCartney practical medical microbiology*. 14th edition, Churchill livingstone, London, pp. 53-94 (1996).
98. Ueda S., Yamaguchi M., Iwase M., and Kuwabara Y.: Detection of emetic *Bacillus cereus* by real-time PCR in foods. *Biocontrol. Sci*., **18**, 227-232 (2013).
99. Kim H.-Y., Kwak I.-S., Hwang I.-G., and Ko G.: Optimization of methods for detecting norovirus on various fruit. *J. Virol. Methods*, **153**, 104-110 (2008).
100. Gyawali P., Ahmed W., Jagals P., Sidhu J., and Toze S.: Comparison of concentration methods for rapid detection of hookworm ova in wastewater matrices using quantitative PCR. *Exp. Parasitol.*, **159**, 160-167 (2015).
101. Gorham J.: Hard foreign objects in food as a cause of injury and disease: A review. *Foodborne Disease Handbook*. Marcel dekker, New York, pp.615-626 (1994).
102. Keener L.: Chemical and physical hazards: the "other" food safety risks. *Food Testing and Analysis Magazine*; http://www.foodsafetyprofessionals.com/keenerhazards.pdf Accessed Oct. 4th, (2018).