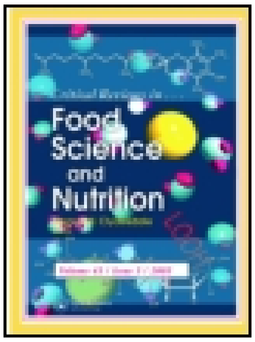

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Most Common Foodborne Pathogens and Mycotoxins on Fresh Produce: A Review of Recent Outbreaks

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Most Common Foodborne Pathogens and Mycotoxins on Fresh Produce: A Review of Recent Outbreaks

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ABSTRACT

Every year millions of people are affected and thousand of them die due to infections and intoxication as a result of foodborne outbreaks, which also cause billions of dollars' worth of damage, public health problems and agricultural product loss. A considerable portion of these outbreaks is related to fresh produce and caused by foodborne pathogens on fresh produce and mycotoxins. Escherichia coli O104:H4 *outbreak, occurred in Germany in 2011, has attracted a great attention on foodborne outbreaks caused by contaminated fresh produce, and especially the vulnerability and gaps in the early warning and notification networks in the surveillance systems in all around the world. In the frame of this paper, we reviewed the most common foodborne pathogens on fresh produce, traceback investigations of the outbreaks caused by these* *pathogens, and lastly international early warning and notification systems including PulseNet International and Rapid Alert System for Food and Feed (RASSF) aiming to detect foodborne outbreaks.*

Keywords: Foodborne pathogens, fresh produce, epidemiology, traceback, notification, RASFF, PulseNet

INTRODUCTION

Fresh produce is an essential raw material for food manufacturers and it offers a wide range of vitamins, nutrients and fibre to consumers demanding healthy food (Carlin, 2007). Fresh produce consists of fruits, vegetables, herbs, and seeds and nuts, which can be in form of whole, prepared (pre-cut or reduced in size), ready to eat (requiring no preparation before consumption) and/or dressed (pH controlled or not) (Goldburn, 2009). The fresh produce is started to be produced and consumed in greater quantities since 1980s as a result of increasing demand of healthy food mostly in high income countries when compared to other agricultural products (FAOSTAT, 2012; Huang, 2004). Therefore, an important portion of recent foodborne outbreaks has been tracebacked to the consumption of contaminated fresh produce with pathogens in all around the world as a result of epidemiological studies (Gorny, 2006)., Mycotoxins - although rarely causing foodborne outbreaks via fresh produce -are still a risk factor for foodstuff of plant origin, especially for seeds and nuts, in underdeveloped countries (Forsythe, 2010).

In the frame of this paper we reviewed: (i) the most common foodborne pathogens and mycotoxins found on fresh produce; (ii) epidemiological studies and traceback investigations of the outbreaks caused by contamintade fresh produce in the last two years; (iii) international early warning and notification networks including PulseNet International and Rapid Alert System for Food and Feed (RASSF).

The most common foodborne pathogens and mycotoxins found on fresh produce

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The fresh produces have both adavantages and disadvantages against pathogen contamination. For example, fresh produces with shells, waxy cover and low pH values prevent microbial contamination (Jay *et al*., 2005), but some with high moisture and nutrient content, and natural openings (stomata, lenticels) are suitable habitate for pathogens (Carlin, 2007; Forsythe, 2010). Contamination of fresh produce with pathogens may occur from farm to fork; during the pre-harvest phase in the field through contaminated seeds, water, soil, dust, insects, or during the post-harvest phase including handling, processing, transportation and preparation phase through contaminated water or cross contamination (equipment, surfaces, handlers, etc.) due to lack of basic hygiene standards (Adams and Moss, 2000; Gorny, 2006; Forsythe, 2010). In terms of foodborne outbreaks related to fresh produce, it is important to notice that if contamination occurs in the production phase or in the first steps of postharvest handling, the risk of cross contamination of pathogens due to distribution of the produce will be much higher than expected (Gorny, 2006).

Because fresh produce is usually consumed raw or is not heat treated for elimination of pathogens, before consumption (Ribot *et al*., 2008), reducing contamination of pathogens from the produce becomes a great challenge. Although there is no possible way to completely eliminate microbial foodborne pathogens from fresh produce, there are available methods to reduce pathogens from fresh produce: physical (brushing, rinsing), chemical (hypochlorite, acidified sodium chlorite, chlorine dioxide, bromine, iodine, trisodium phosphate, quarternary ammonium compounds, acids, hydogen peroxide, and ozone), and biological (using microbial antagonists as a biocontrol agent) methods together with irradiation method (Parish et al., 2003).

For example, amount of microbial pathogens on the fresh produce can be reduced 10-100 fold via treatment with disinfectants. Simillarly washing fresh produce with hot water can also provide considerable amonut of reduction (Forsythe, 2010).

In humans, foodborne pathogen infections manifest itself as symptoms ranging from milder complications like diarrhea, vomiting, abdominal pain, fever, headaches and muscle aches (DôAust and Maurer, 2007; McClane, 2007; Seo and Bohach, 2007) to severe health problems like enterotoxin poisoning, autoimmune complications, meningitis, septicemia, bloddy diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS), and also miscarriage in pregnants (Adams and Moss, 2000; Meng *et al*., 2007; Robins-Browne, 2007; Lampel and Maurelli, 2007; Johnson, 2007, Swaminathan *et al*, 2007). Foodborne pathogens, disscussed within the frame of this article, do not have target populations, however, the risk groups which are primarily affected by these infections can be listed as pregnant women, infants, elderly and immuno-comprimised adults (Forsythe, 2010).

Concerning fresh produce, *Salmonella* spp, pathogenic *Escherichia coli*, *Shigella* spp, *Yersinia* spp, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium* spp. are of fundamental importance.

***Salmonella* species**

Salmonella is a facultatively anaerobic, non-spore forming, rod-shaped and usually motile genus causing two types of illnesses: nontyphoidal salmonellosis which has milder symptoms like diarrhoea, fever, vomiting and abdominal pain, and typhoid fever which has more severe smyptoms (Forsythe, 2010). *Salmonella* species are generally mentioned by the names of serotypes, which are already determined to be over 2500 (Behling *et al.*, 2010). Humans and animals are primary reservoirs of *Salmonella* species and *Salmonella* is abundant in nature (Jay *et al*., 2005). *Salmonella* species can contaminate fresh produce, especially the vegetables, both during the production phase through water, soil, insects or other animals, which are contaminated with faecal matter (Adams and Moss, 2000), and during the preparation phase through, cross contamination (equipment, surfaces, handlers, etc.) (Jay *et al.*, 2005). Moreover, depending on the serotype, *Salmonella* species can be tolerant to low or high temperatures, and extreme acidic environments, and consequently may not be eliminated due to inappropriate storage or handling conditions (DôAoust and Maurer, 2007).

Pathogenic *Escherichia coli*

E. coli is a non-spore-forming, short, facultative anaerobe, rod-shaped bacterium which is a part of normal bacterial gut flora of humans and represents a diverse group of bacteria, in which small pathogenic groups can cause human illnesses ranging from diarrhea to hemolytic uremic syndrome (HUS) (Behling *et al.*, 2010). There are six pathogenic groups of *E. coli* which are grouped according to their symptoms of illness and mechanism of pathogenesis: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), and diffusely adherent *E.coli* (DAEC) (Forsythe, 2010). However, the strains of pathogenic *E. coli* can easily acquire virulence traits and cause wide range of diseases (Meng *et al*., 2007). Among these pathogenic groups, the most severe diesases like bloddy diarrhoea, thrombic thrombocytopenic purpura (TTP), hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) are caused by EHEC group, which also includes the Shiga-toxin-producing *E.coli* (STEC) serotypes (Forsythe, 2010). As primary reservoirs are ruminants, especially cattle, this pathogen can contaminate fresh produce during both during the production phase through contaminated water with intected animal faeces, or during the preparation phase through cross contamination (equipment, surfaces, handlers, etc.) (Forsythe, 2010). Because fresh produce is generally consumed raw and heat treatment cannot be applied to its products, cooking before consumption is not an option for elimination of pathogenic *E.coli*, however, irradiation is a more useful method to eliminate these pathogens that are also tolerant to low pH (Meng *et al*., 2007).

In terms of EHEC, EPEC and EAggEC infections, infants/children under and the elderly are at more risk (Forsythe, 2010). Especially children under the age of six can develop HUS due to EHEC infection. In some cases, HUS may develop chronic kidney disease and failure (Weiss and Schmidt, 2011).

***Shigella* species**

Shigella is a non-motile, non-spore forming, rod-shaped genus comprised of highly infectious species (*S. sonnei*, *S. boydii*, and *S. flexneri*, *S. dysenteriae*) causing illnesses ranging from diarrhea to bacillary dysentery or enterotoxin/shigatoxin related hemolytic uremic syndrome (HUS) with a low infectious dose (Forsythe, 2010). The only source of *Shigella* is infected humans, therefore fresh produce can be contaminated with *Shigella* species through water or insects, which are contaminated with human feces (Adams and Moss, 2000), or during the preparation phase through cross contamination (Jay *et al.*, 2005). As these pathogens can survive in environmental conditions including dried surfaces, low temperatures and low pH, they can easily survive in fresh produce products, however, they can be reduced with ionizing radiation and sodium hypochlorite (Lampel and Maurelli, 2007).

***Yersinia* species**

Yersinia, a rod-shaped genus including species, includes speies (especially *Y. enterocolitica* and *Y. pseudotuberculosis*) causing illnesses, ranging from diarrhea, vomiting and abdominal pain to fever and bloody diarrhea in humans through fresh produce (Jay *et al*., 2005). *Yersinia* is abundant in nature and can contaminate fresh produce both during the production phase through contaminated water, soil or insects or during the preparation phase through cross contamination (equipment, surfaces, handlers, etc.), due to lack of basic hygiene standards (Forsythe, 2010). Because *Y. enterocolitica* is tolerant to low temperatures (below 4^{o}C) and extreme alkaline conditions (pH 4-10), it can easily survive and contaminate frozen products due to inappropriate storage conditions (Robins-Browne, 2007). *Y. enterocolitica* can be inactivated by ultraviolet irradiation (Butler *et al*.,1987) and aqueous ozone (Restaino *et al*., 1995).

Listeria monocytogenes

L. monocytogenes is a rod-shaped, facultative, non spore-forming, motile bacterium and a foodborne pathogen of high public health concern. *L. monocytogenes* causes illnesses ranging from muscle aches and diarrhea to meningitis, sepsis and miscarriage in pregnants (Behling *et* *al.*, 2010). Among 13 serotypes of *L. monocytogenes*, serotypes 1/2a, 1/2b, and 4b cause most of the foodborne outbreaks concerning listeriosis (Swaminathan *et al*., 2007). *L. monocytogenes* is abundant in nature and can contaminate fresh produce both during the production phase through soil, decaying vegetation, water, animal feces, sewage, silage and many other environmental sources or during the preparation phase through cross contamination (equipmentôs, surfaces, handlers, etc.) (Adams and Moss, 2000; Jay *et al.*, 2005). Moreover, because this pathogen is tolerant to high salt concentrations and low temperatures, they can easily survive in food-manufacturing environments and contaminate frozen products due to inappropriate storage conditions (Behling *et al.*, 2010). Treatment with acids is a useful tool to eliminate *L. monocytogenes* when compared to other sanitizing agents (Swaminathan *et al*., 2007).

L. monocytogenes primarily cause severe outcomes in pregnant women, newborns, the elderly and people who have weak immune system (Forsythe, 2010). As mortality rate of listerosis is high in comparison with other foodborne pathogens on fresh produce, and during pregnancy infection can be transmitted from mother to placenta, resulting in abortion, stillbirth and permature birth, listerosis is a crucial threat to the pregnants and newborns (Adam and Moss, 2000).

Staphylococcus aureus

S. aureus is a spherical, facultative anaerobic, and non-motile bacterium causing food poisoning via staphylococcal enterotoxins. Thirteen of staphylococcal enterotoxins are identified (Jay *et al.*, 2005). Symptoms of Staphylococcal food poisoning ranges from nausea to abdominal pain, vomiting and diarrhea in humans (Forsythe, 2010). Primary reservoirs of *Staphylococci* are humans and animals (nose, throat and skin of them) but they can also be found in air, dust, sewage and water in nature (Behling *et al.*, 2010). Contamination of fresh produce may occur both during the production phase through water or direct contact with food by human/animal carriers, or during the preparation phase through cross contamination (equipmentôs, surfaces, handlers, etc.) (Jay *et al.*, 2005). Being one of the most resistant non-spore forming foodborne pathogens to environmental conditions, this organism is tolerant to drying and high salt concentrations (Seo and Bohahch, 2007). Enterotoxins of this pathogen are also resistant to high temperatures, therefore enterotoxins cannot be eliminated easily by cooking, but *Staphylococcus aureus* contamination can be prevented by simple personal hygiene measures or by keeping foodstuff at either high or low temperatures (60 ^{0}C and above, 7.2 ^{0}C and below) before consumption (Forsythe, 2010).

***Clostridium* species**

Clostridium is an anaerobic (aero tolerant), rod-shaped, spore-forming genus causing illnesses ranging from watery diarrhea to botulism in humans (Jay *et al.*, 2005). Among four clinically important Clostridium species (*C. botulinum, C. perfringens, C. difficile, C. tetani*), food poisoning cases are caused by only *C. perfringens* and *C. botulinum* (Forsythe, 2010). While enterotoxin producing *C. perfringens* is responsible for milder symptoms, botulinum-producing species (*C. butyricum*, *C.baratii*, and especially *C. botulinum*) are responsible for more serious symptoms and cause a neuroparalytic illness called botulism (Johnson, 2007).

There are seven different botulinum toxins that are grouped according to their toxic antigenicity and mentioned by group names from A to G (A, B, E and rarely F cause illnesses in humans) (Forsythe, 2010). As botulinum toxins are the most toxic substances known and there is not much thing to do after the toxin is absorbed, early diagnosis is crucial (Adams and Moss, 2000). Symptoms of botulism intoxication, which generally start to be seen between 12-36 hours after consumption of toxins, can be listed as double vision, vomiting, nausea, headache, dryness in throat and nose and respiratory failure (Forsythe, 2010). Critical point is, onset of botulism symptoms can extend up to eight days and respiratory and heart failure generally occurs in 1-7 days in fatal cases (Adams and Moss, 2010).

The spores of these species are very common in nature, especially in soil, and can contaminate fresh produce both during the production phase through water, soil and dust or during the preparation phase through cross contamination (equipmentôs, surfaces, handlers, etc.) (Adams and Moss, 2000; Jay *et al*., 2005). Preventing contamination of spores of *Clostridium* species is not always possible, however, spores of these pathogens can be partially inactivated by ionizing irradiation, chlorine and hydrogen peroxide when thermal processing is not possible (Johnson, 2007).

Clostiridium species are tolerant to low oxygen levels; they can easily survive in home-canned fruits and vegetables or the vegetables stored in oil (e.g. garlic) and cause botulinum poisoning due to toxin production inside the body after the spores are taken into the body with food

(Johnson, 2007). As this is a spore-forming genus, *Clostridium* species can survive in challenging environmental conditions like drying, heating, and toxicity (Jay *et al*., 2005).

Clostridium species do not have a target population in terms of foodborne botulism. Although in some regions of the world human foodborne botulism incidence is common, outbreaks due to *Clostridium* species are not common in the EU and USA (Johnson, 2007).

Mycotoxins

Mycotoxins cause epidemics in humans via food products as secondary metabolites produced by some fungi in the end of exponential growth phase (Jay *et al*,, 2005). Mycotoxins may invade the produce via many contamination pathways during pre-harvest phase through seeds, soil and air or during the post-harvest especially in storage (Forsythe, 2010). While the low pH content of fresh produce, especially fruits, is a disadvantage for bacterial pathogens, it is a opportunity for fungi invasion (Moss, 2008). On the contrary, relatively higher pH values in vegetables makes them susceptible for bacterial pathogens (Adams and Moss, 2010).

Presence of fungi on fresh produce does not always result in mycotoxin contamination; however, environmental factors may trigger mycotoxin formation (Drusch and Ragab, 2003). For example, if pulses, nuts and oilseeds are not stored at appropriate temperatures, which keep these products at certain water content, fungal activity may occur (Adams and Moss, 2000). If contamination occurs, complete elimination of mycotoxins from food products is not possibble (Bennett and Klich, 2003). However, mycotoxins in foods can be partially degraded by physical and chemical

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methods as well as irradiation (Jay *et al*,, 2005). For example, mycotoxin content of the fruits can be reduced with washing and sorting in the post-harvest phase (Drusch and Ragab, 2003).

In humans, uptake of mycotoxins via food products may results in mycotoxin poisoning which manifest itself as symptoms including milder ones like diarrhoea, abdominal pain, other gastrointestinal problems and more severe complications like cancer (Adams and Moss, 2000). Susceptibility of humans against mycotoxins may change not only by type of exposure (acute or chronic), but also by age, sex and overall health of the person exposed (Forsythe, 2010). For example, males are generally more susceptible than the female against aflatoxins (Adams and Moss, 2000).

Concerning fresh produce, aflatoxin, ochratoxin A, citrinin and patulin are of fundamental importance whereas zearalenone, fumonisins and deoxynivalenol are main problems in terms of cereals.

Aflatoxin

Aflatoxins are a group of natural toxins mainly produced by certain strains of *Aspergillus flavus, A. parasiticus* and *A. nominus* under appropriate storage and environmental conditions especially in warmer regions of the world (tropics and subtropics) due to high temperatures and high humidity (Adams and Moss, 2000). These toxins are mentioned by the group names B_1, B_2, G_1, and G_2, which cause mild to severe complications in humans such as liver failure (Hocking, 2007; Forsythe, 2010). Aflatoxins often contaminate food products due to their high toxicity

(Bhatnagar *et al*., 2008). This mycotoxins is mostly produced in in fresh produce and other raw agricultural commodities including nuts, figs, dried fruits, cereals and oilseeds (Jay *et al*,, 2005).

Ochratoxin A

Ochratoxin A (OTA) is a natural toxin mainly produced by *Aspergillus ochraceus* and *Penicillium verrucosum* under appropriate storage and environmental conditions especially in temperate regions of the world (Moss, 2008). As a nephrotoxin, OTA may cause mild to severe complications in humans including various kidney problems due to acute or chronic exposure and the dose exposed (Bennett and Klich, 2003). This mycotoxin mostly found in products of tropical and subtropical origin including cereals (such as maize), cocoa, coffea and soybeans, but can also be present in spices, dried fruit, and nuts (Adams and Moss, 2000).

Citrinin

Citrinin is a natural nephrotoxin, which is mainly produced by certain strains of *Penicillium* (*P. citrinum* and *P. verrucosum*) and *Aspergillus* (*Aspergillus ochraceus)* under poor storage conditions (Adams and Moss, 2000). As a nephrotoxin, citrinin may cause mild to severe complications in humans including kidney problems and yellow rice fever due to acute or chronic exposure and the dose exposed (Bennett and Klich, 2003). Citrinin can be found in fresh produce including fruits, herbs, beans, and spices and in other raw agricultural commodities such as cereals (EFSA, 2012).

Patulin

Patulin is a natural toxin mainly produced by certain strains of P*enicillium* (especially *P. expansum*), *Aspergillus* and *Byssochlamys* under adverse storage conditions (Adams and Moss, 2000). Toxicological effects of patulin have been studied in many animals, however, complications of exposure to patulin due to food products in humans is still unclear (Jackson and Dombrin-Kurtzman, 2006). Patulin is especially found in fruits due to their low pH levels such as apples, pears, grapes, bananas, peaches and pineapples and it may also be present in seeds of plants (Jackson and Dombrink-Kurtzman, 2006; Jay *et al.*, 2005).

Epdemiological studies and traceback investigations of the outbreaks caused by contaminated fresh produce in the last two years

According to investigations of outbreaks, which have been conducted in all around the world in the last two years, the most common known foodborne pathogens on fresh produce (Table 1) appear as Enterohemorrhagic *E. coli* serotypes (O104:H4 and O157:H7), and *Salmonella enterica*. Less commonly, *L. monocytogenes* and *Shigella sonnei* are found to be causative agents of these outbreaks. The most common sources of these foodborne pathogens on fresh produce appeared as sprouts, seeds and nuts. Between the periods of November 2010 ï November 2012, totally 5191 people were infected with and 95 people died due to the outbreaks caused by fresh produce, which was contaminated with foodborne pathogens. Concerning these outbreaks, it is noteworthy that newborns, children, elderly and pregnants are prone to the infections caused by foodborne pathogens on fresh produce. Highest mortality rate was seen in the outbreaks caused by *L. monocytognes*, followed by Enterohemorrhagic *E. coli* (EHEC) and *Salmonella* species.

Although pathogenic *E. coli* and *Salmonella* species infected thousands of people and totally caused 65 deaths in eighteen outbreaks, *L. monocytogenes* caused 30 deaths in just one outbreak by infecting 146 people.

In terms of mycotoxins, the outbreaks causing public health problems have declined after strict limits were set by both national and international legislation with the efforts of Europen Commission and institutions of United Nations like Food and Agricultural Organization and World Health Organization (Adams and Moss, 2000). However, still there can be occasional outbreaks caused by mycotoxins due to inappropriate storage and environmental conditions. Mycotoxins, especially aflatoxin, are a food safety problem in underdeveloped countries where detection and surveillance programs for foodborne diseases are not well established (Bhatnagar *et al*., 2008).

Concerning the mycotoxins, there has been no new outbreaks occurred in the last two years, therefore, we examined the outbreaks occurred in the period of last ten years. In the two outbreaks occurred in Kenya and Brazil, aflatoxin and citreoviridin appear to be the causative agents in this period of time. The sources of these outbreaks were contaminated cereals (rice and maize). Due to these outbreaks, totally 837 people were intoxicated and 157 people died due to the two outbreaks caused by mycotoxin containment. Among the causative agents of these outbreaks, aflatoxins are detected as the most dangerous mycotoxin, having the highest mortality rate.

Extensive surveillance studies in the field revealed the magnitude and underlying reasons of these outbreaks caused by mycotoxin containment. According to these studies, it was seen that due to inappropriate storage conditions mycotoxin contamination occurs and causes hundreds of peoplesô death in mostly the underdeveloped countries where food inspection and food safety measures cannot be implemented (CDC, 2004; Alves *et al.*, 2010). Strosnider *et al*. attributed the reasons of this malfunction of food monitoring systems in underdeveloped countries to lack of resources, technology and infrastructure (Strosnider *et al*., 2006).

Fresh produce is generally consumed as mixed salads or garnish. Identifying the source product inside the mixed food is the first challenge for the epidemiologists in fresh produce related outbreaks (Berger *et al*., 2010). Among the outbreaks summarized in this review, it was seen that public health investigatiors used some common methods in order to rise to this challenge and trace back the source. They used web-based questionnaires and repeated interviews with patients, whose infection is confirmed in laboratory, in order to find the soruce of outbreaks. In some cases, in addition to interviews and questionnaires, electronic purchase records of patients, which were investigated in the related shopping with their permission. After confirmation of the same agent, causing disease in patients, in the suspected food source from questinnaires, the source pathogen is announced to the public via public health institutions. Besides the biochemical analysis and phenotypic characterization, subtyping methods are commonly used to detect the agent of outbreak from the suspected source. Although different subtyping methods are used to find the contaminated produce, pulsed-field gel electrophoresis (PFGE) is the gold standard molecular method for subtyping foodborne pathogens.

In most of the outbreaks we examined, trace back studies showed that the fresh produce item was contaminated during production phase at a single producer company. In all of the outbreaks occurred in the USA, after the contamination pathway is determined, the responsible company voluntarily recalled the product from the market. Apart from the USA, voluntary recall of the contaminated product was seen in other countries too, for example in Norway, fresh produce was voluntarily recalled in the situation of an outbreak caused by contaminated fresh produce.

In the USA, epidemiological and traceback investigations performed in close collaboration with The United States Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA) and public health officials. For every outbreak, recall information is publicly announced and consumers and people who sell/serve the food are warned about the contaminated product and provider company via the website of CDC (Cdc.gov, 2012). Also, even the contamination pathway is unclear; FDA may issue an import alert announcing that import of the contaminated product is banned unless the importer proves that their product is not contaminated (Fda.gov, 2011).

Like in other individual countries in the world, public health officials in the Member States of European Union (EU) and other European Economic Area (EEA) countries inform the local and national food safety authority in the case of a foodborne outbreak. However, when there is a situation of a cross-border outbreak in EU, national public health officials inform other EEA countries of the current outbreak via the Epidemic Intelligence Information System (EPIS) of the

European Centre of Disease Prevention and Control (ECDC) and Early Warning Response System (EWRS) with the request to report any similar cases (Guglielmetti, 2006). In the coming year, ECDC release annual epidemiological and surveillance reports of these outbreaks on certain communicable diseases including foodborne pathogens on fresh produce (Ecdc.europa.eu, 2012).

Scientific publications of national or global public health agencies about the recently occurred outbreaks form an important part of foodborne disease outbreak surveillance and provide guidance for the next outbreaks. Health agencies of the USA and EU provide such reports annually or singly after each outbreak in scientific journal of their governmental agencies. However, to obtain such data for every country in the world is almost impossible due to lack of a fully functional global disease surveillance system.

Here we reviewed two major outbreaks occcured in 2011 as examples of epidemiological studies in fresh produce from different countries.

E.coli O104:H4 *Outbreak in 2011*

Epidemiology: According to the Robert Koch Institute (RKI, 2011), 3842 people infected (2987 cases of bloody diarrhea, 855 cases of HUS) with the outbreak strain of *E. coli* O104:H4 and 53 people died as a result of the infection in the fifteen countries including Denmark, France, Greece, Great Britain, Luxembourg, Netherlands, Norway, Austria, Poland, Sweden, Spain, Czech Republic, Canada, Switzerland, USA (RKI, 2011). Illnesses began on May 1st and lasted on July 26th, 2011. After July 5th that the active phase of the outbreak ended, only sporadic cases of HUS occurred due to secondary transmission (RKI, 2011). 68 per cent of the HUS patients and 57 % of the non-HUS (acute gastroenteritis) patients were female.

Traceback: On May 22nd, 2011, Germany notified the World of this outbreak through EU Early Warning and Response System (EWRS) (WHO, 2011). Four days after the outbreak, the source was announced to be the Spanish cucumbers (ECDC, 2011). On June 6th, in order to investigate the outbreak, a team sent to Berlin including experts from European Center for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Commission (ECDC, 2011). After a small outbreak in France, where the cases had no recent travel history to Germany, EFSA declared the source as a specific lot of fenugreek seeds imported from Egypt, which were subsequently used for sprout production both by a horticultural farm in Lower Saxony and by private individuals (EFSA, 2012). Although where and how the contamination occurred had not been detected, EC temporarily banned the import of these products on the same day that source was declared (European Commission, 2011). Moreover, the outbreak strain could not be detected in any of the food samples of plant origin probably because of inadequacy of detection methods or inability of enrichment of bacteria (Winter, 2012).

Beginning from early June, various special reports were published on the outbreak in Germany. Firstly, in a technical report, ECDC and EFSA published the characteristics of the outbreak strain (*E.coli* STEC O104:H4), which is very rare and has never been reported in food before (ECDC-EFSA, 2011). 2 days later, World Health Organization declared this outbreak as the biggest ever occurred in Europe, the second biggest ever seen on worldwide, and the most deadly EHEC outbreak ever reported due to its size and virulence (WHO, 2011). After the outbreak was over, ECDC listed the unusual features of the outbreak as: adults being at higher risk, women developing more HUS than men, the outbreak strain as being highly virulent and standard methods to test for STEC used in most EU laboratories could not detect this rare serotype (ECDC, 2011). Finally, RKI published a final report clarifying the details of the outbreak (RKI, 2011) as: repeated online questionnaires were performed by RK in order to monitor the consumption patterns (especially fresh produce) and hygiene behavior of the consumer, more than 50 % of the cases were notified to the RKI within two days and 75 % were notified within four days after receipt of the report at the local health authority and incubation period of the infection was calculated as 8 days on average (RKI, 2011).

Although the contamination pathway of the source is still unclear (The Federal Institute for Risk Assessment/BfR, 2011), the outbreak in Germany solely caused unaccountable losses in economy due to misinformation about the source, the delay in both determination of the source pathogen and its hosts and in reporting the cases (ECDC, 2011) and trade bans (STEC Workshop Reporting Group, 2011). According to experts, besides this outbreak was one of the most serious outbreaks according to the number of cases and deaths, it is the most costly outbreak in history and the overall cost was estimated to be in 0.5-3.5 billion US dollars (Food Safety News, 2012).

Listeriosis Outbreaks in 2011

Epidemiology: According to Centers for Disease Control and Prevention (Cdc.gov, 2011), totally 30 people died, 1 woman had a miscarriage, 142 people (99 %) hospitalized and totally 146

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infected (3 newborn, 4 pregnant) with any of the four outbreak-associated strains of *Listeria monocytogenes* were reported from 28 states. CDC publicly announced detailed information about these outbreaks: illnesses began on July 31st and lasted on October 27, 2011. Ill people range in age from 1 to 96 years, with a median age of median 77 years. 58 per cent of the ill people were female. Dead people ranged in age from 48 to 96 years, with a median age of 82.5 years.

Trace back: After the investigations performed in collaboration with CDC, FDA and public health officials, this multistate outbreak of *L. monocytogenes* was linked to whole cantaloupe grown at Jensen Farms' production fields in Granada, Colorado. The pathogen was identified on cantaloupes collected from grocery stores and from ill persons' homes (Cdc.gov, 2011). Using the data from PulseNet USA, DNA fingerprint patterns of *L. monocytogenes* stains were obtained through diagnostic testing with PFGE (Cdc.gov, 2011).

During the interviews, among the 140 ill people, 131 (94 %) reported consuming cantaloupe in the month before illness onset (Cdc.gov, 2011). In the environmental assessment report released on October 19, 2011 FDA denoted that producer company of the contaminated cantaloupes was Jensen Farms (FDA, 2011). After the trace back investigations, FDA announced that firstly Jensen Farms voluntarily recalled the cantaloupes concerning with this outbreak and then Carol's Cuts LLC - a Kansas food processor - and Fruit Fresh Up Inc. - a New York food processor - recalled product containing cantaloupes originated from Jensen Farms (Fda.gov, 2011).

International early warning and notification networks

There are useful tools to take necessary precautions to prevent cross-border foodborne outbreaks in the world where the whole food system has globalized. Rapid Alert System for Food and Feed (RASFF) is an effective regional early warning system achiving this goal via alerts, information notifications and border rejections. RASFF, a communication system, was established to inform EU Member States and provide them the opportunity to take action more rapidly against the risks detected in relation to food or feed. Apart from rapid communications, RASFF publishes annual reports. In the Annual Report of 2011, it was denoted that a total of 3812 original notifications (market notifications as alerts and information notifications; and border rejections) were made through the RASFF (alert 635, information for follow-up 573, information for attention 744 and border rejection notification 1860). This report also denotes that 37 % of these notifications were related to fresh produce, herbs and nuts (fruits and vegetables 670, herbs and spices 198 and nuts, nut products and seeds 526). It is evident from the RASFF statistics that the notifications related to fresh produce constitutes an important percentage of the total notifications in the recent years (Figure 1). Another fact can be derived from the RASFF statistics that notifications regarding pathogenic microorganisms are raising but, on the contrary, notifications regarding mycotoxins are gradually decreasing in the recent years (Figure 2). The rise in the notifications concerning pathogenic microorganisms in fresh produce is mainly due to *Salmonella* spp in fruits and vegetables, herbs and spices. (RASFF Annual Report, 2011).

Listeria monocytogenes (in fish and diary products), pathogenic *Escherichia coli* (in sea food), *Bacillus cereus* (in pasta, rice, herbs and spices), noroviruses (in sea food), *Clostridium* *botulinum* (in canned/preserved foods), and *Campylobacter* (in meat and fresh vegetables) followed *Salmonella* spp in the 2011 notifications.

On the other hand, aflatoxin has been dominating the mycotoxin related notifications in RASFF in the last decades, while deoxynivalenol (DON), fumonisins, ochratoxin A, patulin, zearalenone and citrinin have been subject to less number of notifications. The decreasing trend in mycotoxins is mainly due to the decrease in aflatoxin containment in spices, nuts and nut products in the recent years (RASFF Annual Report, 2011).

There are also surveillance networks to detect and trace of foodborne diseases, located at health agencies in individual countries. The best example is PulseNet in the USA. In 1996, PulseNet was established in USA as a national molecular subtyping network for foodborne disesase surveillance under the coordination of the Centers for Disease Control and Prevention (CDC). Right now, PulseNet is a national network of public health and food regulatory agency laboratories coordinated by the Centers for Disease Control and Prevention (CDC) in the USA. After the success of informal collaborations between the PulseNet USA and PulseNet Canada, the need for international cooperation was realized and beginning with PulseNet Canada different regional/national networks namely PulseNet Latin America & Caribbean, PulseNet Europe, PulseNet Africa, PulseNet Middle East and PulseNet Asia Pacific were established and started to cooperate under the umbrella of PulseNet International (Swaminathan *et al*., 2006). Although real-time data sharing is not effective among all the regional/national networks, PulseNet International aims to provide an effective collaboration and communication on early warning of outbreaks, emerging pathogens and bioterrorist acts between these networks (Cdc.gov, 2012).

Apart from these systems, there are some other global networks of UN Institutions assisting communication among national public health authorities. One of them, International Food Safety Authorities Network (INFOSAN), is a joint initiative of Food and Agricultural Organization of UN (FAO) and World Health Organization (WHO). INFOSAN aims to provide rapid communication among national food safety authorities in order to disseminate the information about emergency situation via publishing information notes and alerts (WHO, 2012). In case of an emergency, INFOSAN collaborates with The Global Outbreak Alert and Response Network (GOARN) of World Health Organization in order to acquire techincal and operational assisstance which is readily available in the Member States of the network (WHO, 2012). INFOSAN also supports with PulseNet International to the goal of a more effective global food borne disease surveillance (INFOSAN, 2009).

CONCLUSIONS

Consumption of fresh produce has been increasing since 1980s on year-by-year basis, therefore, outbreaks caused by contaminated fresh produce has been in an upward trend. On the contrary, the outbreaks causing public health problems via fresh produce have declined after strict limits were set by both national and international legislation.

Epidemiological studies of these cross-border outbreaks caused by fresh produce contaminated with foodborne pathogens in the recent years and especially the *E. coli* O104:H4 outbreak in 2011 revealed vulnerability and gaps in the early warning and notification networks in the national and global surveillance systems. Basic weakness of available international networks appears as delay in detection and real-time data sharing. For instance, as a global network, power of PulseNet International is providing a database based on using the same molecular method for subtyping, however, real-time data sharing is only effective between PulseNet USA and PulseNet Canada while this network has five other regional or national networks.

In order to fill these gaps and establish a fully functional global surveillance system including rapid early warning and detection networks for both known and emerging pathogens on food products, improving available regional networks like Rapid Alert System for Food and Feed and global networks such as INFOSAN and PulseNet International is crucial.

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Table 1 Recent Outbreaks Caused by Foodborne Pathogens on Fresh Produce

Date/Duration	Country	Name of Disease	Pathogen	Source	Number of Case	Voluntary Recall
October ï November 2012	USA	HUS/diarrhea	*E.coli* (STEC) O157:H7	Pre-packaged organic spinach and spring mix	33 infected,13 hospitalized, 2 HUS (5 states), 63% female	+
Ref: http://www.cdc.gov/ecoli/2012/O157H7-11-12/index.html						
July ï September 2012	USA	Salmonellosis	*Salmonella* Braenderup	Mango	127 infected, 33 hospitalized (15 states),55% female	+
Ref: http://www.cdc.gov/salmonella/braenderup-08-12/index.html						
July - September 2012	USA	Salmonellosis	*Salmonella* Typhimurium *Salmonella* Newport	Cantaloupe	261 infected, 94 hospitalized, 3 dead (24 states), 55% female	+
Ref: http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/index.html						
July ï August 2012	Japan	Diarrhea	*E. coli* O157	Pickled Chinese	110 infected, 7 dead	

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				cabbage	Many were above 80 years old	
Ref: http://www.yomiuri.co.jp/dy/national/T120821004270.htm http://www.bangkokpost.com/news/asia/308364/contaminated-pickles-kill-seven-in-japan						
April ï June 2012	Canada	Diarrhea	*E. coli* O157	Romaine lettuce	55 infected, 18 hospitalized,	
Ref: http://www.cbc.ca/news/canada/new-brunswick/story/2012/06/29/nb-e-coli-miramichi-lettuce-1033.html						
December 2011 ï February 2012	UK, Germany	Salmonellosis	*Salmonella* Enteritidis	Ready-to-eat sliced watermelon	1 dead	
Ref: http://www.dailymail.co.uk/health/article-2095387/Watermelons-linked-salmonella-outbreak-killed-left-50-ill.html						
December 2011 ï March 2012 (2 months)	USA	Diarrhea	*E.coli* (STEC) serotype O26	Raw clover sprouts	29 infected, 7 hospitalized (11 states), 89% female	+
Ref: http://www.cdc.gov/ecoli/2012/O26-02-12/index.html						
October -	USA	HUS/	*E.coli*	Romaine	58 infected, 33	+

November 2011 (2 months)		diarrhea	(VTEC) serotype O157:H7	lettuce	hospitalized, 3 HUS (from 9 states), 59 % female	
Ref: http://www.cdc.gov/ecoli/2011/ecoliO157/romainelettuce/032312/index.html						
July - October 2011 (4 months)	France, Germany, Denmark	HUS /diarrhea/ bloody diarhea	*E.coli* (EHEC) serotype O104:H4	Foodborne (unknown)	11 hospitalized (France 8, Germany 2, Denmark 1)	
Ref: http://www.eurosurveillance.org/images/dynamic/EE/V17N04/art20065.pdf						
September-October 2011 (2 months)	Denmark, Germany, Austria	Salmonellosis	*Salmonella* Strathcona	Tomato	40 hospitalized (Denmark 40, Germany 14, Austria 1) 60% female	
Ref: http://www.ssi.dk/English/News/News/2011/Salm%20imported%20tomatoes.aspx						
July - October 2011 (2 months)	USA	Listeriosis	*Listeria monocytogenes*	Cantaloupe	146 infected , 142 hospitalized, 30	+

					dead, 1 miscarriage (from 28 states), 58% female, majority was over 60 years old	
Ref: http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html						
October 2011 (1month)	Norway	Shigellosis	*Shigella sonnei*	Imported fresh basil	46 hospitalized 50% female/male	+
Ref: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20007						
August 2011 (1 month)	USA	Salmonellosis	*Salmonella* Enteritidis	Pine nuts	43 infected, 2 hospitalized (from 5 states), 60% female	+
Ref: http://www.cdc.gov/salmonella/pinenuts-enteriditis/111711/index.html						
January ï August 2011 (8 month)	USA	Salmonellosis	*Salmonella* Agona	Fresh imported papayas	106 infected, 10 hospitalized (from 25 states),	+

					58% female 39% under 5 years old	
Ref: http://www.cdc.gov/salmonella/agona-papayas/082911/index.html						
December 2010-July 2011 (8 months)	UK	Diarrhea/ HUS	*E.coli* serotype O157 (Phage type 8/PT8)	Leeks and potatoes	250 infected, 74 hospitalized (4 HUS), 1dead, 69% female 40% under 16 old	
Ref: http://www.guardian.co.uk/world/2011/sep/30/ecoli-outbreak-uk-250-ill						
May-July 2011 (3 months)	EU/EE A (14 countri es)	HUS Non- HUS	*E.coli* serotype O104:H4	Sprout/Seed related	3842 infected (855 HUS, 2987 Non-HUS), 53 dead (35 HUS, 18 non-HUS),	
Ref: http://ecdc.europa.eu/en/publications/Publications/1106_TER_EColi_joint_EFSA.pdf http://www.euro.who.int/__data/assets/pdf_file/0009/144981/EHEC_outbreak_10_June_2011.pdf http://ecdc.europa.eu/en/aboutus/organisation/Director%20Speeches/201109_MarcSprenger_STEC_ICAAC.pdf http://www.rki.de/EN/Home/EHEC_final_report.pdf?__blob=publicationFile						
April ï June	USA	Salmonel	*Salmonella*	Alfalfa	21 infected, 3	+

2011 (3 months)		losis	Enteritidis	sprouts and spicy sprouts	hospitalized (from 5 states) 71 % female	
Ref: http://www.cdc.gov/salmonella/sprouts-enteritidis0611/062611/index.html						
February ï April 2011 (3 months)	USA	Salmonel losis	*Salmonella* Panama	Cantaloupe	20 infected, 3 hospitalized (from 10 states), 65% male	+
Ref: http://www.cdc.gov/salmonella/panama0311/062311/index.html						
December 2010 - February 2011 (3 months)	USA	Diarrhea / Bloody diarrhea	*E.coli* serotype O157:H7	In-shell hazelnuts	8 infected, 4 hospitalized (from 3 states), 75% male	+
Ref: http://www.cdc.gov/ecoli/2011/hazelnuts0157/index.html						
November 2010 - February 2011 (4 months)	USA	Salmonel losis	*Human Salmonella* serotype I 4,[5],12:i:-	Alfalfa / Spicy sprouts	140 infected, 34 hospitalized (from 26 states), 63% female	+
Ref: http://www.cdc.gov/salmonella/i4512i-/021011/index.html						

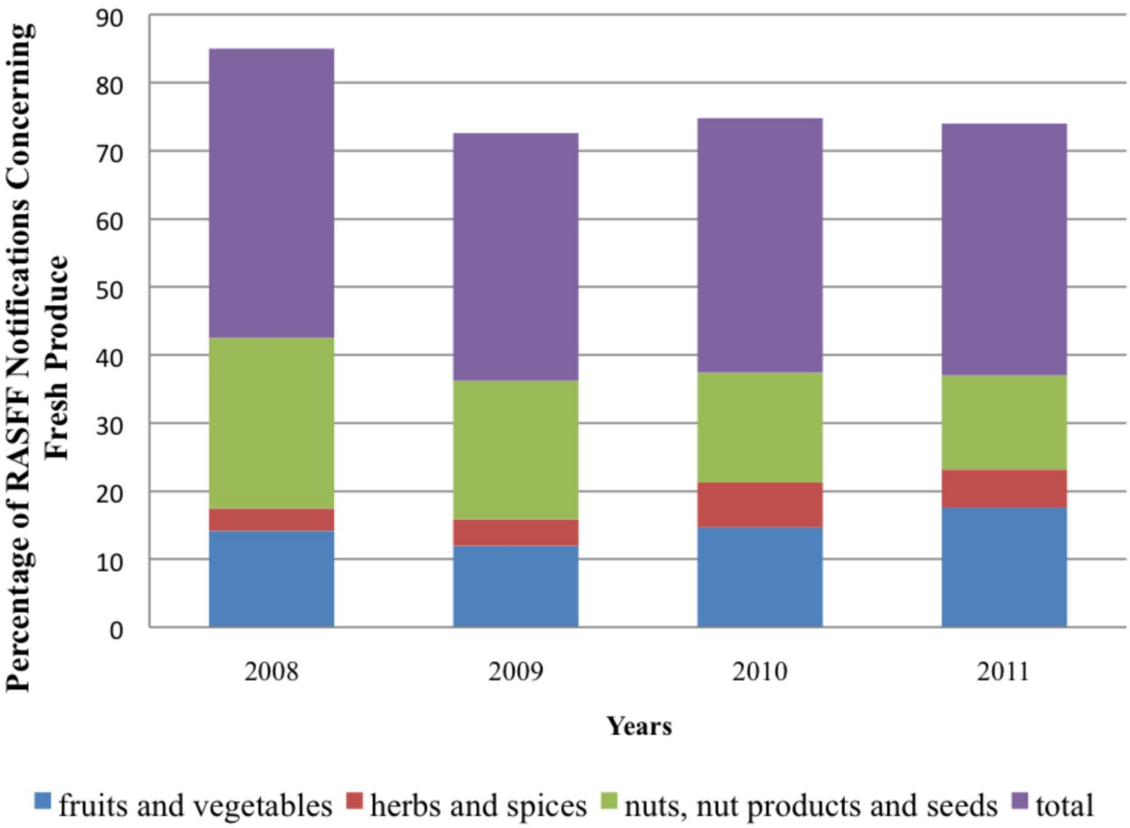

Figure 1 - Percentage of RASFF Notifications concerning fresh produce (2008-2011)

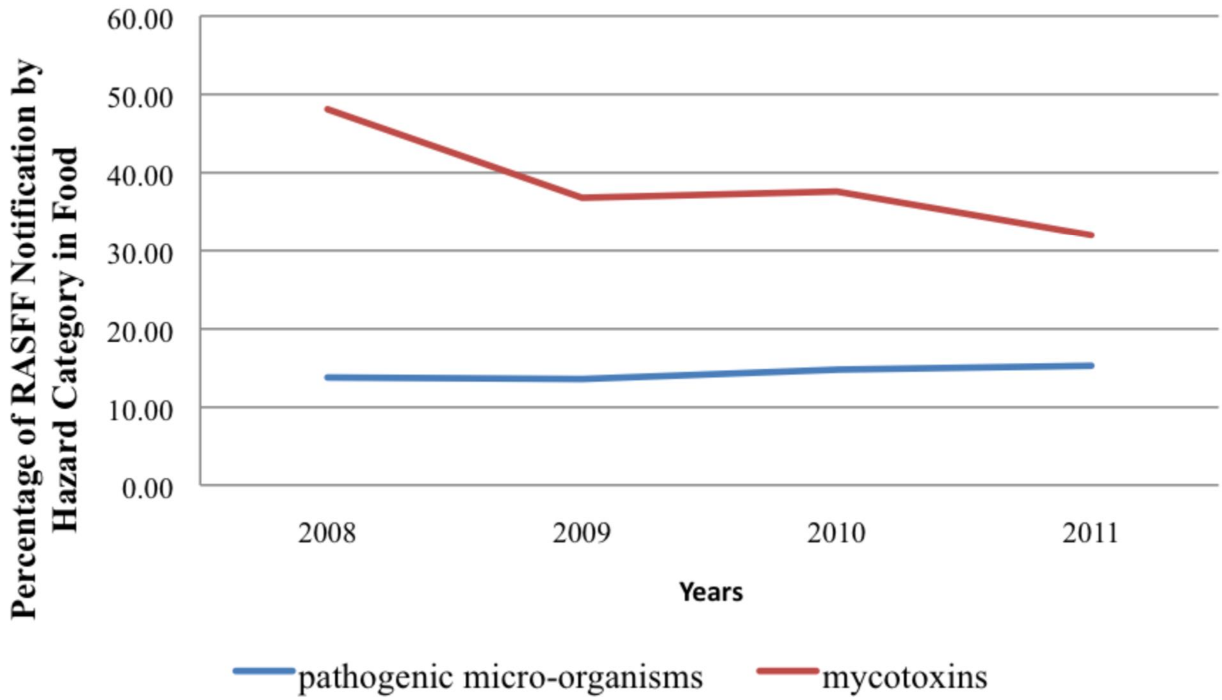

Figure 2 - Percentage of RASFF Notifications by Hazard Category in food (2008- 2011)