Outbreaks and factors influencing microbiological contamination of fresh produce

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Abstract

Fresh fruits and vegetables are nutritionally well-recognized as healthy components in diets. The microbiological foodborne outbreaks associated with the consumption of fresh produce have been increasing. *Salmonella* spp., *E. coli* O157:H7, *Staphylococcus aureus*, *Campylobacter* spp. and *Listeria monocytogenes* are the most common pathogens that contaminate fresh produce. This review discusses recent foodborne outbreaks linked to fresh produce, factors that affect microbiological contamination and measures that could be adopted to reduce the foodborne illnesses.

Keywords:

fresh produce; *E. coli* O157:H7; *Staph. aureus*; *Salmonella* spp.; antibiotic resistance

INTRODUCTION

Microbiological food safety has always been a focus of the food industry and public health agencies since foodborne pathogens cause many illnesses and deaths worldwide. According to Eurosurveillance editorial team [1], a total of 5,048 outbreaks of foodborne illnesses occured in the European Union (EU) in 2011. The most common pathogens responsible were *Campylobacter* (220,209 cases) followed by *Salmonella* (95,548) and pathogenic *E. coli* (9,485). New food consumption trends indicate that people are interested in fresh produce because of the many essential nutrients for health and wellbeing are present in them. Vegetable and fruit production have been increasing and their average consumption per capita has risen from 45 to 54% from 1976 to 2009 in the USA [2] and this trend has continued.

The number of the foodborne outbreaks linked to the consumption of fresh produce have also increased, with an estimated 14.8% in 1998 to 22.8% in 2007 in the USA [3]. Table 1 lists some of the recent foodborne outbreaks

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associated with fresh produce recorded between 2013 and 2016. It is interesting to note that the type of pathogen responsible for each outbreak was different (Table 1). Only some selected examples are used to demonstrate the diversity of pathogens involved in fresh produce contamination. Outbreaks of *Yersinia pseudotuberculosis* in New Zealand in 2014 and *Cyclospora cayetanensis* in the USA in 2013 and 2014 were two of the most significant outbreaks associated with fresh produce. However, *Salmonella* and *Listeria monocytogenes* are emerging as two major bacterial pathogens of concern in terms of fresh produce safety (Table 1).

Contamination can occur at any point, from the farm to plate. According to World Health Organization (WHO) [4], a hazard can exist in production systems due to several factors: post-harvest practices, water, local environment, fertilizer, worker health and hygiene, and consumption patterns and practices. Cross-contamination components to *Listeria* levels in fresh produce was reported a significant factor [97]. As fresh produce is normally consumed raw or with minimum proccesing, it is important to keep the microbial load of fresh produce as low as possible to prevent foodborne illnesses. This review discusses the contamination types of fresh produce, recent outbreaks (2013-2016), frequency of foodborne outbreaks (in selected countries) and factors affecting the microbial food safety of fresh produce as well as future implications of microbiological contamination of fresh produce.

Table 1. Foodborne outbreaks associated with fresh produce (2013 – 2016)

Year/Country	**Produce involved**	**Pathogen/cases**	**References**
2016/Australia	Rock melon	*Salmonella* Hvittingfoss (97 cases)	[88]
2016/Australia	Pre-package lettuce	*Salmonella anatum* (144 cases)	[33]
2016/UK	Imported salad	*E. coli* O157 (161 cases, 60 hospitalised, 2 deaths)	[34]
2016/USA	Packaged salads	*L. monocytogenes* (19 cases, 19 hospitalised, 1 death)	[89]
2015/Australia	Imported frozen strawberries	Hepatitis A virus (19 cases)	[90]
2015/USA	Imported cucumber	*Salmonella* Poona (>900 cases, 204 hospitalised, 6 deaths)	[91]
2014/USA	Prepackaged caramel apples	*L. monocytogenes* (32 cases, 31 hospitalised, 6 deaths)	[94]

2014/USA	Mung bean sprouts	*L. monocytogenes* (5 cases, 5 hospitalised, 2 deaths)	[94]
2014/New Zealand	Fresh vegetables (exact source remains unknown)	*Yersinia pseudotuberculosis* (334 cases, 65 hospitalised)	[64]
2014/UK	Lettuce, cucumber	Enteroinvasive *E. coli* O96 (50 cases)	[65]
2014/UK	Salads	*Salmonella singapore* (4 cases)	[65]
2014/USA	Raw clover sprouts	*E. coli* O121 (19 cases, 8 hospitalised)	[21]
2014/USA	Coriander	*Cyclospora cayetanensis* (304 cases, 7 of 183 hospitalised)	[21]
2013/USA	Bean sprouts	*S. enteritidis* (87 cases, 27 hospitalised)	[21]
2013/USA	Imported cucumber	*Salmonella* (84 cases, 17 hospitalised)	[21]
2013/USA	Imported pomegranate seeds	Hepatitis A virus (165 cases, 69 hospitalised)	[21]
2013/USA	Salad mix	*Cyclospora cayetanensis* (631 cases, 50 hospitalised)	[21]
2013/USA	Imported cucumber	*E. coli* O157:H7 (33 cases, 11 hospitalised)	[21]
2013/UK	Watercress	Vero cytotoxin-producing *E. coli* O157	[65]

MICROBIAL CONTAMINATION OF FRESH PRODUCE

According to Westrell et al. [5], *Salmonella* infections caused 151,995 human cases of salmonellosis in 2007, the second most prevalent in the 27 EU Member States and the four European Free Trade Association (EFTA) countries. In 1995, there was a large outbreak of *Salmonella* in the USA and Finland [6], originating from the seeds of alfalfa sprouts. Similarly, Mohle-Boetani et al. [7] reported seven outbreaks of *Salmonella* in the USA between 2000 to 2002 related to the consumption of raw mung bean sprouts. Greene et al. [8] reported a *Salmonella* outbreak (510 cases in 2002) due to contamination of tomatoes by *S. newport*, a rare strain of *Salmonella*. Fresh produce-associated salmonellosis outbreaks are becoming common and new strains or serotypes were identified in the recent outbreaks. In 2015, *Salmonella* Poona was identified in an outbreak linked to cucumber (>900 cases, 204 hospitalised, 6 deaths) in USA [91]. This year so far two *Salmonella* outbreaks in Australia have been attributed to fresh produce; *Salmonella* anatum outbreak (144 cases) linked to pre-package lettuce and *Salmonell*a Hvittingfoss (97 cases) linked to rock melons [33, 88].

The incidence of food poisoning caused by *E. coli* O157:H7 have been on the increase. In 1995, *E. coli* O157:H7 was detected in 40 patients (13 hospitalized) in the USA, and 70% of these patients reported having consumed leaf lettuce [9]. In 1996, *E. coli* O157:H7 poisoning occurred most likely from white radish sprouts with 7,996 people ill, 398 hospitalized, and three deaths [10]. *E. coli* O157:H7 has also been traced to sprouts [11], cantaloupes, apples and leaf lettuce [9, 12]. Wachtel and Charkowski [13] reported that 72 cases of *E. coli* O157:H7 poisonings in the USA in 1999 were from shredded iceberg lettuce. Westrell et al. [5] reported a total of 2,905 cases of *E. coli* O157:H7 poisonings in 27 EU Member States and the four European Free Trade Association (EFTA) countries.

In 2016, a national level outbreak of a rare strain of *E. coli* O157 associated with mixed salad leaves was investigated by the Public Health England (PHE). This specific strain of *E. coli* causes infection with symptoms ranging from mild diarrhoea to bloody diarrhoea with severe abdominal pain. This recent outbreak had a total of 161 cases (England 154, Wales 6 and Scotland 1) with 60 hospitalisations and 2 deaths [34].

Staph. aureus is also a major cause of foodborne diseases. This bacterium generally exists in nasal mucosa, skin and hair of animals and sometimes produce enterotoxins [15]. Of the total food poisoning cases in the UK, 1 to 6% between 1969 and 1981, 0.5 to 1% between 1982 and 1990 were caused by *Staph. aureus* [16]. This foodborne pathogen was attributed to 1.5% of all outbreaks (N = 2530) from 1992 to 2009 in UK and ranked as the sixth most common bacterial causes during this period [35]. One of the common origins of *Staph. aureus* is from meat products but any product can be contaminated by *S. aureus* because it can be passed on to produce from the skin of workers [16]. It is commonly detected in fresh vegetables, with 56.9% of salad vegetables purchased from street vendors in India [17] and all vegetables purchased from five different shops in Bangladesh [18] contaminated with *Staph. aureus*.

L. monocytogenes is a well-known pathogen for which mortality is sometimes higher than for other pathogens [2]. According to Lim et al. [19], 23 listeriosis notifications were reported in New Zealand in 2010, 31 people hospitalized and seven died. Eighty-five percent of the listeriosis illnesses were estimated to be foodborne [14]. In 2010, the number of New Zealand listeriosis poisonings were higher in females, the most vulnerable being women over 70 years old (deaths occurred in those over 70 years old) [19]. The most dangerous factor was underlying illness (68.8%) or receiving immunosuppressive drugs (52.9%). The trend of the number of the outbreaks has fluctuated over the years. Lim et al. [19] reported that *L. monocytogenes* isolates were confirmed in 22 New Zealanders in 2010. In 2011, during a multi-state listeriosis outbreak in the USA caused by *L. monocytogenes* in cantaloupe, 99% of patients were hospitalized and 33 patients (22%) died in the USA [20]. Prevalence of *Listeria* In fresh produce was reported in several investigation [3, 32, 71, 96, 97] Fresh produce-associated listeriosis outbreaks are regularly occurring in the USA: 2014, mung bean sprouts (5 cases, 5 hospitalised, 2 deaths); 2014, caramel apple (32 cases, 31 hospitalised, 6 deaths); and 2016, packaged salads (19 cases, 19 hospitalised, 1 death) [89, 94].

Figure 1 shows the contribution of the different of pathogens associated with foodborne outbreaks of fresh produce in the USA between 1998 and 2012 [21]. According to the Center for Disease Control and Prevention (CDC) [21] in the USA, between 1998 and 2012, 889 outbreaks out of 16,576 were related to fresh produce but the pathogen type was identified only in 699 cases. As seen in figure 1, norovirus was the most common, followed by *Salmonella* and Enteropathogenic *E. coli*. Norovirus and hepatitis A viruses are the most important foodborne viruses where fresh produce has been recognised as a transmission vehicle. This article primarily discusses bacterial pathogen in fresh produce. Some recent publications have addressed the problem of foodborne viruses in fresh produce [86, 87]. Table 2 lists selected studies on microbial contamination of fresh produce or cut vegetables. All these studies showed similar results for the level of contamination with aerobic plate count (APC) and the presence of pathogens.

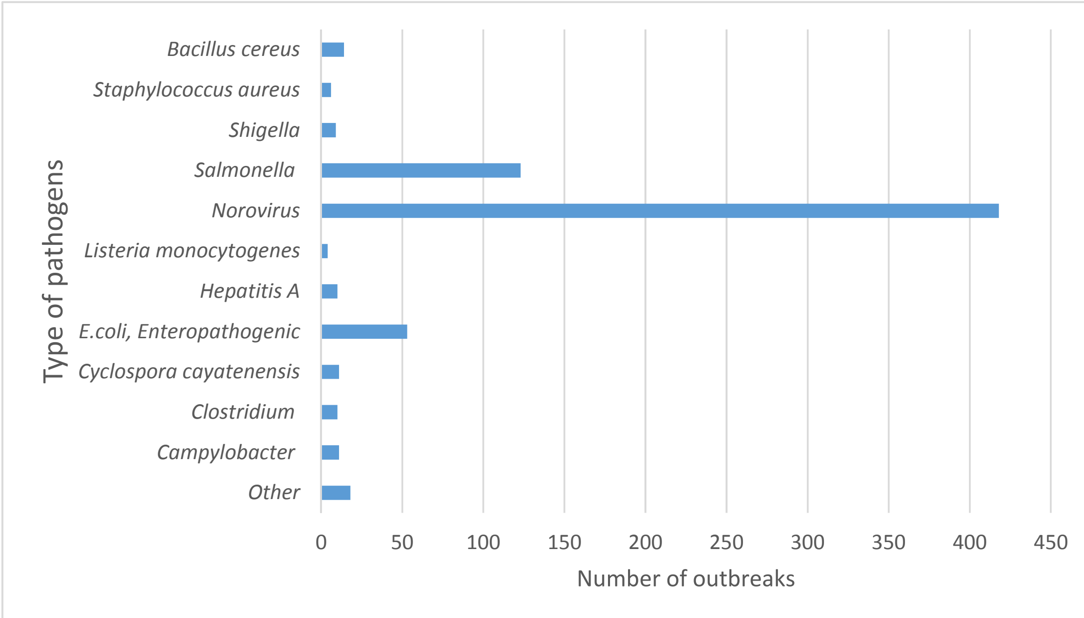

Figure 1. Foodborne pathogens linked to outbreaks caused by contaminated fresh produce in the USA between 1998-2012 [21]

Table 2. Selected reports of microbial contamination of fresh produce

Produce	**Microbial contamination level**	**References**
Raw salad vegetables (parsley, lettuce, radish)	*E. coli* 0.7–7 $\log_{10}$ CFU/g; Total coliform 1.69–8.16 CFU/g *L. monocytogenes* (14%); *Staph. aureus* (45.5%)	[95]
Whole vegetables	*L. monocytogenes* 5.7%.	[32]
Mix salad, ready-to-eat (RTE) salads and sprouts	APC 10^7-10^8 CFU/g; *E. coli* 40%; *Salmonella* 1.3%; and *L. monocytogenes* 0.7%. No *Y. enterocolitica*; *E. coli* O157:H7; and *Campylobacter*.	[66]
Whole vegetables	APC 0–7.4 $\log_{10}$ CFU/g; *E. coli* 0–3.8 CFU/g.	[67]
Fruits and vegetables, packed RTE vegetables	APC 10^0-10^{10} CFU/g; coliform 10^0-10^{10} CFU /g; yeast and mold 10^1-10^9 CFU/g.	[68]
Whole vegetables, RTE vegetables, mixed salads and mixed lettuce	*Salmonella* 0.75% of the whole vegetables; *L. monocytogenes* 1.61% of the whole vegetables; and 0.29% in RTE samples. No *E.coli* O157:H7.	[69]
Whole vegetables, herbs and fruits	APC 10^4-10^8 CFU/g; *L. monocytogenes* 0.64%. No *Salmonella*; *Shigella*; or *E. coli* O157:H7.	[70]
Whole vegetables	*E. coli* 8.94%; *L. monocytogenes* only in organic 1.12%. No *E. coli* O157:H7; *Salmonella*.	[71]
Whole vegetables	APC 10^6-10^7CFU/g; *E. coli* 41.5%- organic and 40%-	[72]

	traditional. No *Salmonella.*	
Mix vegetables and whole vegetables	*Aeromonas* 34%. No *E. coli; E. coli* O157:H7; *Salmonella; Listeria; or Campylobacter*.	[73]
Whole vegetables	APC 10^5CFU/g traditional, 10^6 CFU/g organic; *E. coli* 12.9% traditional and 22.2% organic. No *E. coli* O157:H7; *L. monocytogenes; or Salmonella.*	[74]
Whole vegetables	Not satisfactory *E. coli; Listeria.* No *Campylobacter; E. coli* O157:H7; *or Salmonella.*	[75]
Whole vegetables and fruits	APC 10^1-10^9 CFU/g. No *E. coli*; or *Salmonella.*	[76]
Whole vegetables	APC 10^5-10^6CFU/g.	[39]
Whole vegetables, sprouts and fruits	APC 10^9-10^{10} CFU /g; *E. coli* 39.2%; *Staph. aureus* 58.3%; and *Salmonella* 28.3%.	[17]
Whole vegetables	*E. coli* O157:H7 0.11%; *L. monocytogenes* 0.11%; and *Salmonella* 0.38%.	[3]

FREQUENCY OF FRESH PRODUCE RELATED FOODBORNE OUTBREAKS

According to the Center for Science in the Public Interest (CSPI) [22], of 4,638 outbreaks (117,136 cases) of foodborne illnesses in the USA from 1998 to 2007, 57 to 70% of them could not be traced to the contamination source. The most frequent source was seafood (838 outbreaks) followed by fresh produce (684 outbreaks), poultry (538 outbreaks) and pork (200 outbreaks) [22]. Vegetables contributed to 33% (228 outbreaks) and about 50% (345 outbreaks) to dishes of produce including salads [22]. In New Zealand, 716 food poisoning outbreaks occurred in 2012, 13.3% of which were from leafy vegetables > root vegetables (10%) > fruits and nuts (6.7%) > stalk vegetables (3.3%) [23]. The most common pathogen was Norovirus (27%) followed by *Salmonella* spp. (20%) and *Campylobacter* spp. (17%)[23]. Table 3 shows the frequency of the foodborne outbreaks linked to fresh produce contamination between 2002 and 2012.

In the USA, the number of fresh produce outbreaks have fluctuated but not declined (Table 3). On average, the USA had 57 outbreaks due to fresh produce contamination each year. In Japan the frequency of foodborne outbreaks (bacteria, viruses and chemicals) related to fresh produce declined by 33% between 2002 and 2012 [24] (Table 3). Table 3 also presents the data for fresh produce (vegetables, fruits and nuts) in New Zealand [25]. On average, New Zealand now report 3 times more fresh produce linked outbreaks compared to in 2002. In general, it is hard to highlight a specific trend in the incidence of such outbreaks between 2002 and 2012.

However, in 2012, there were 5 times as many outbreaks than a decade ago. It is apparent from above that fresh produce is a considerable contributor towards foodborne outbreaks throughout the world.

Table 3. Frequency of foodborne outbreaks linked to fresh produce contamination between 2002 and 2012 for USA [21]; Japan [24]; and New Zealand [25]

Year	Frequency of fresh produce-associated outbreaks in selected countries		
	USA	**Japan**	**New Zealand**
2002	53	12	2
2003	45	9	2
2004	81	5	2
2005	51	8	8
2006	78	8	4
2007	47	10	1
2008	63	6	6
2009	41	5	10
2010	58	5	10
2011	49	9	6
2012	60	8	10
Yearly average	**57**	**8**	**6**

Figure 2 shows the number of *Salmonella* positive samples in fresh produce in the EU from 2011 to 2012 [26]. The number of *Salmonella* positive samples differed between countries, probably because each country sets its own safety guidelines to manage food safety of fresh produce. In 2011, only 3 countries (Denmark, Germany and Belgium) in the EU had *Salmonella* positive samples, but in 2012, 8 countries (Denmark, Germany, Hungary, Netherlands, Poland, Romania, Belgium and Spain) had *Salmonella* positive samples. Fresh produce industry is not very well-regulated for microbiological safety and testing. European commission for Agriculture and Rural Development published microbiological criteria for foodstuffs in November 2005 (Commission Regulation (EC) No 2073/2005, Official Journal L 338 of 22 December2005, p. 1). Regular testing of fresh, uncut and unprocessed vegetables and fruits, excluding sprouted seeds against the criterion is considered not useful in normal circumstances. However, testing of *E. coli* and *Salmonella* for processed fresh produce (pre-cut fruit and vegetables, unpasteurised fruit and vegetable juices) and *Salmonella* for sprouted seeds is required to ensure safety. This indicates management of food safety was not that effective in the EU member countries at this stage. An effective management system for fresh produce could be set up to correct this.

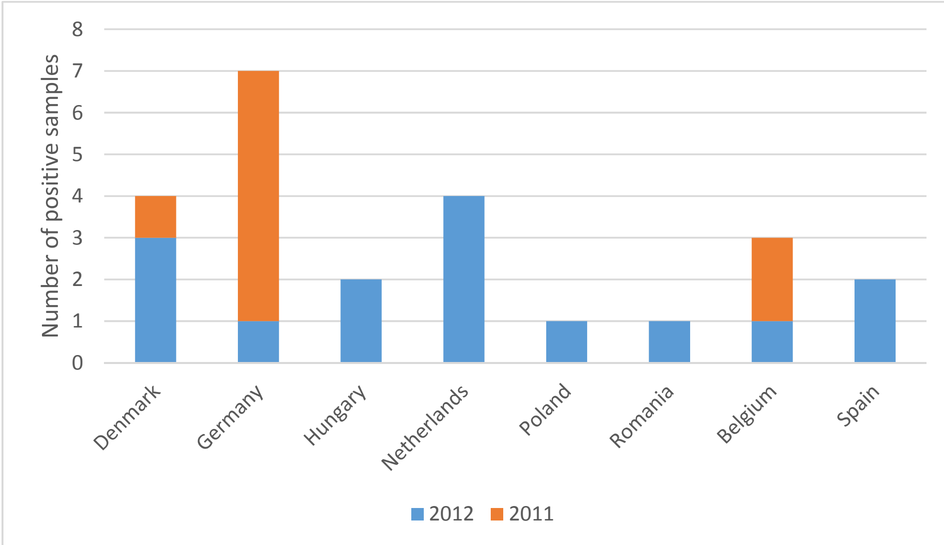

Figure 2. Number of *Salmonella* positive samples in fresh produce in the European Union countries from 2011 to 2012 [85]

FACTORS THAT AFFECT MICROBIAL CONTAMINATION OF FRESH PRODUCE

There are many intrinsic and extrinsic factors that favour microbial growth. Firstly, the moisture content in fresh produce is approximately $a_w = 0.97 – 1.00$, which is favourable for microbial growth. The optimum moisture content for growth of *Salmonella* spp., enterohemorrhagic *E. coli* and *Campylobacter* spp. is 0.99, for *Staph. aureus* it is 0.98 and the minimum moisture content for *L. monocytogenes* is 0.92 [27]. The problem is that it is difficult to reduce the moisture content of fresh produce unless it is processed. Secondly, pH is an important factor because each microbe type has an optimal pH for its growth. According to Koutsoumanis and Sofos [28], *E. coli* O157:H7 and *S.* Typhimurium can tolerate even acidic pH (to pH 5.0), while *L. monocytogenes* grows only to pH 6.0. According to the US Food and Drug Administration (FDA) [27], pH 4.6 is about the minimal pH for growth of most pathogens. However, there are some pathogens, such as *C. botulinum*, that can withstand acidic pH as low as pH 4.2. Several researchers including Kim and Hung [29] used electrolyzed oxidizing water to change the surface pH of blueberries and this was effective in reducing the microbial load. A third factor is the presence of different types of nutrients in fruits and vegetables, such as proteins, fat, minerals and vitamins. Sugars and proteins are the most common energy source for pathogens. However, some pathogens require special nutrients to grow. For example, *Staph. aureus* requires biotin for its growth [30], while the growth of *Salmonella* increases in the presence of iron (compared to a non-iron environment) [31]. Since each vegetable has a different nutrient composition, analyzing optimal pathogen growth on different types of vegetables by analyzing their nutrients requires further study.

Oxidation-reduction (or redox) potential (ability of materials to oxidize or reduce) of the food also influences the pathogen growth of. According to Snyder [36], oxidation-reduction potential varies between food types and has a profound effect on the growth of some microorganisms such as *C. botulinum* [37]. The redox potential of a food is controlled by many factors, such as pH, packaging and types of ingredients in a food. Normally the redox potential of fresh produce is low [27]. Guentzel et al. [38] analyzed the effect of redox potential on *E. coli*, *S.* Typhimurium, *Staph. aureus*, *L. monocytogenes* and *Enterococcus faecalis* using electrolyzed oxidizing water at a near-neutral pH condition and reported that all microbial counts were markedly reduced. This method has no side effects and therefore is an excellent way to improve food safety of fresh produce without application of chemicals or antimicrobial agents.

Antimicrobial agents which originate from natural or artificial materials have a strong impact on pathogen growth. Sirsat and Neal [39] used vinegar (2.5% acetic acid) on *E. coli* and *Salmonella* and reported that it significantly reduced microbial count (2 to 3 $\log_{10}$CFU/g). Similarly, Medina et al. [40] reported that the effect of vinegar, olive oil and several beverages including wine were effective in controlling the growth of certain pathogens (*S. enteritidis*, *L. monocytogenes*). According to Gálvez et al. [41], many lactic acid bacteria can produce bacteriocins through lactic fermentation, which can act as an antimicrobial agent. Vidhyasagar and Jeevaratnam [42] reported that the bacteriocin produced from one of the lactic acid bacterial species (*Pediococcus pentosaceus* VJ13) has antimicrobial effects on *Mycobacterium smegmatis*, *Klebsiella pneumonia*, *C. perfringens* and *Staph. epidermidis*. Since bacteriocins are effective and produce only minimal side effects, investigations into applying it to fresh produce would be helpful. In addition, the US FDA [27] reported that there are many types of artificial antimicrobial agents (preservatives or additives) such as nitrates and nitrites, sulfur dioxide, acetic acid and nisin that could be used to control or eliminate foodborne pathogens in fresh produce.

Food processing conditions also affect pathogen growth. Firstly, the packaging or product environment has a significant impact on pathogen growth. According to Sun et al. [43], chlorine dioxide gas significantly reduced the total bacterial count, *E. coli*, yeast, and mould concentration on fresh blueberries. Simon et al. [44] reported that an optimum concentration of gases (low oxygen and high carbon dioxide concentrations) could be determined for each product to minimize microbial growth. Thompson [45] reported that oxygen, nitrogen and carbon dioxide are the most important gases in a modified atmosphere for pathogen control in fresh produce. Nitrogen has antimicrobial properties, oxygen is effective in controlling anaerobic microbes and carbon dioxide, which can reduce pH if dissolved in the liquid portion of a food, has an inherent antimicrobial activity. The recommended percentage of these gases differs for each fresh produce type. This is an area that requires further investigation.

Temperature is one of the important factors for bacterial growth. Koseki and Isobe [46] conducted an experiment on the growth of *E. coli* O157:H7 and *Salmonella* spp. in lettuce at different time periods (0 h to 120 h) and exposure temperatures (5, 10, 15, 20, and 25^{o}C). Bacteria increased with both time and temperature. The optimal temperature for each of the following pathogens are: *S. typhi* - 35 to 37^{o}C [47], *E. coli* O157:H7 - 37^{o}C [48], *Campylobacter* - 42^{o}C [49], *Staph. aureus* - 37^{o}C and *L. monocytogenes* - 37^{o}C [50]. There is one exception as ESR [51] reported, the optimal temperature of some strains of *C. botulinum* (those that are proteolytic, mesophilic and produce toxins A, B or F, group 1) is 35-40^{o}C, but for those strains that are non-proteolytic, mesophilic and produce B, E or F type toxins (group 2) it is 18-25°C. In addition, the minimum temperature at which *C. botulinum* group 2 strains can grow is only 3°C, whereas the minimum growth for group 1 strains is 10°C [51]. *C. botulinum* is an anaerobic bacterium that causes a disease called botulism (which can be fatal). This disease tends to affect those who consume canned products stored in a cold place because inside of the canned product is an anaerobic environment. Date et al. [52] reported 3 outbreaks of botulinum poisoning caused by consumption of canned vegetables produced at home in the USA. According to these authors [52], 38% of botulism cases occurring between 1999 and 2008 in the USA were from canned products produced at home although commercial canned products have also caused botulinum poisoning. In 2007, an outbreak of botulism in the USA was caused by canned hotdog chili sauce [53]. In order to achieve better food safety two factors are important i.e., optimal thermal processing for different kinds of products and/or packaging to kill the vegetative cells and pH adjustment (slight acidic) of the product to prevent spore germination.

According to Shalini and Singh [54], factors that inhibit pathogen growth are called ‘hurdles’. ‘Hurdle technology’ (a combination of ‘hurdles’) should be used to prevent pathogen growth. Hurdles include the factors mentioned above, such as different processing methods. For fresh produce such as lettuce, radish sprouts and apples, a combination of calcium oxide and sonication was effective in lowering the pathogen load (*E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium) more than sonication or calcium oxide alone [55]. Similarly, Brown et al. [56] used a combination of chlorine and lactic acid bacteria to inhibit *E. coli* O157:H7 and *C. sporogenes* in ready-to-eat spinach. It was reported that the hurdles were effective in reducing the pathogen load, except in *C. sporogenes* which has the ability to grow at even at low (3.3°C) temperature [56]. In contrast, Ganesh et al. [57] used 'natural agents' as hurdles, such as malic, tartaric and lactic acids and grape seed extract as an electrostatical spray on spinach and lettuce to reduce the *E. coli* O157:H7 load, and found that all agents except tartaric acid were effective. As there is the possibility of using a combination of hurdles, more research could be directed to investigate the most effective hurdle combination for each fresh produce.

Table 4. Strategies that have been tested for postharvest applications

Produce type	**Strategies**	**References**
Leafy greens	Bacteriophage cocktail, BEC8 (10^6 CFU/leaf with the essential oil trans-cinnameldehyde (0.5% v/v).	[63]
Fresh produce (lettuce, radish sprout and apple)	Calcium oxide (2% CaO) and sonication (10min at a frequency of 20 kHz).	[55]
Lettuce	Wash with calcinated calcium (the heated scallop shell powder).	[77]
Carrots	Mildly heated (45°C), slightly acidic electrolyzed water with low available chlorine (23 mg/L).	[78]
Cauliflower	8% Salt; 0.3% Citric acid; 300 ppm Potassium metabisulphite; and 300 ppm Sodium benzoate. Store at 5-7°C.	[79]
Broccoli	Irradiation with UV-C light (8 kJ m^{-2}) and then heating (45°C, 3h in air oven) .	[80]
Paprika	Wash in 1% calcium chloride and 6% calcium ascorbate combined with 50°C water temperature for 2 min.	[81]
Cantaloupe	Hot water (75°C, 1min); and gaseous ozone (10,000 ppm, 30min).	[82]
Tomato	Humidified flow of ozone-enriched air (4 ± 0.5 μL^{-1} of ozone for 30 min every 3 h).	[83]
General	Conducting research in fresh produce safety; Implementation of food safety programs; Outreach education for farmers, shippers and processors;	[84]

	Promote the safe and careful handling of fresh produce.	

FUTURE IMPLICATIONS

The number of foodborne outbreaks has been variable in spite of the many measures taken to reduce the incidence. Outbreaks have varied between different countries but here is no obvious trend in the outbreaks related to fresh produce. Recent trends show that people prefer healthy foods including fresh vegetables and fruits. As fresh produce is normally eaten raw, more research is required to reduce the pathogen load on fresh produce. Currently, not many countries have regulations to monitor pathogens in fresh produce. Therefore, guidelines to manage fresh produce to prevent contamination of pathogens should be set and monitored regularly. In addition, effective technologies to manage fresh produce with materials produced from natural agents need to be tested. Examples of recommended strategies to reduce bacterial contamination in fresh produce are listed in Table 4. Although some studies report the use of natural agents as hurdles, there is little information on the combined effects of these agents [58, 59]. Other possible hurdles reported include use of electrolyzed oxidizing water [60], bacteriocins [61], modified atmosphere [62], bacteriophage spray [63] and strict management of temperature and storage times. Publications by Qadri et al. [92] and Mukhopadhyaya and Raghupathy [93] provide further information on the fresh produce contamination and control measures. Fresh-cut fruits and vegetables is one of the growing sectors within fresh produce industry offering convenient products; however, maintaining quality and safety of such product is a major challenge [92]. Several novel preventative approaches including essential oils, green tea extract and biopreservation were proposed to reduce microbial risks. There is a need for adequate processing technologies to ensure fresh-cut produce safety and consumer acceptability [92]. In conclusion to keep fresh produce safe, further studies are required to determine how to manage contamination of pathogens and fresh produce safety throughout the supply chain.

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