

BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Advances in ensuring the microbiological safety of fresh produce

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Introduction

Increasing consumer demand for low-input cultivation and minimal processing has significantly increased the risk of microbiological contamination of fresh produce. This both presents a health risk to consumers and undermines trust in the food supply chain from farm to fork.

This volume reviews our current understanding of the main pathogenic risks to fresh produce, including their epidemiology, genetics and behaviour. The book is split into three parts: Part 1 chapters focus on understanding current pathogenic risks in fresh produce, specifically focusing on *Salmonella*, *Listeria* and *Escherichia coli*. Chapters in Part 2 examine developments in detection and risk assessment of pathogens, such as rapid detection/high throughput screening techniques, modelling pathogen behaviour and microbiological risk assessment for pathogens in fresh produce. Part 3 chapters address improving food safety along the value chain, specifically discussing sources of pathogen contamination, the role of good agricultural practices in preventing contamination, as well as developments in sanitising and packaging techniques. Chapters also discuss the role of good manufacturing practice, hazard analysis and critical control point systems to maintain fresh produce safety and improving safe consumer handling of fresh produce.

Part 1 Pathogenic risks

The book opens with a chapter that focuses on understanding contamination of fresh produce by *Salmonella*. Chapter 1 begins by first reviewing the various plant traits and agricultural practices that aid *Salmonella* association with fruit and vegetable crops. It also discusses the genetic strategies that *Salmonella* employs to compete in the plant niche. The chapter moves on to examine *Salmonella* in various types of produce, such as sprouts and microgreens, root, bulbous and stalk vegetables and leafy vegetables and fresh herbs. It also examines *Salmonella* in fruit, tree nuts and seeds. A section on *Salmonella* strategies in the plant niche is also provided.

The subject of Chapter 2 is advances in understanding and presenting contamination of fresh produce by *Listeria monocytogenes*. The chapter begins by first describing the pathogen's pathogenicity and gene regulation, followed by an overview of recent *L. monocytogenes* outbreaks linked to fresh produce contamination. A section on the sources of fresh produce supply chain contamination is also included, focusing specifically on pre-harvest, harvest and postharvest contamination. The chapter includes a case study on *L. monocytogenes* contamination in cantaloupes, which is then followed by an

overview of current strategies used to control *L. monocytogenes* in the fresh produce supply chain. Sections on *L. monocytogenes* gene regulation, survival mechanisms and its adaptation to various forms of stress are also included. The chapter examines biocontrol of *L. monocytogenes* and also describes the novel processing technologies to control the pathogen.

The final chapter of Part 1 examines advances in understanding contamination of fresh produce by pathogenic *Escherichia coli*. Chapter 3 first describes the pathogenicity and virulence of *Escherichia coli*, then moves on to analyse produce contamination with pathogenic *E. coli*. The chapter then describes the interaction of pathogenic *E. coli* with fresh produce and highlights various produce outbreaks linked to pathogenic *E. coli*. A section on food safety regulations and future actions for *E. coli* contamination is also provided, focusing specifically on the research describing the pathogen's growth and survival in soil and plants and current implications for pathogen control.

Part 2 Detection and risk assessment

Part 2 begins with a chapter that focuses on developments in rapid detection/high throughput screening techniques for identifying pathogens in food. Chapter 4 highlights current conventional methods in place for detecting foodborne microorganisms, focusing on techniques such as culture-based assay and immunoassays. The chapter goes on to review molecular methods such as polymerase chain reaction, loop-mediated isothermal detection, nucleic acid sequence-based amplification, recombinase polymerase amplification, DNA microarray and whole-genome sequencing. This is then followed by an overview of current spectroscopic methods, specifically Raman spectroscopy, Fourier-transform infrared spectroscopy, near-infrared spectroscopy and hyperspectral imaging spectroscopy. The chapter describes mass spectrometry methods such as matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry, optical phenotyping methods such as forward light scattering and flow cytometry and biosensor methods. Sections on the detection of parasites and the advantages and limitations of microbial detection methods are also included.

The focus of Chapter 5 is advances in modelling pathogen behaviour in fresh produce. Chapter 5 begins by first describing methods for modelling spoilage of fresh produce, which is then followed by an overview of current modelling methods for behaviour in fresh produce, focusing specifically on growth modelling and pathogen inactivation. A section on modelling transfer of pathogens during fresh produce processing is also included, highlighting how microbial transfer can be modelled between processing equipment and foods.

The next chapter draws specific attention to advances in quantitative microbiological risk assessment for pathogens in fresh produce. Chapter 6 takes a microorganism and historical perspective to review advances in quantitative microbial risk assessment of fresh produce. The chapter reviews microbial risk assessment research over the past 25 years, specifically focused on *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7 and Norovirus as important pathogens that have all caused outbreaks linked to fresh produce.

Part 3 Improving safety along the value chain

The first chapter of Part 3 reviews advances in understanding sources of pathogenic contamination of fresh produce, focusing specifically on soil and soil amendments. Chapter 7 first discusses pathogen survival in soils containing biological amendments, which is then followed by an examination of *E. coli* and *Salmonella* survival in manure dust. A section on the prevalence of antibiotic resistance genes in manure-amended soils is also provided, followed by that draw attention to outbreaks in fruit and vegetables in 2018 and 2020. The chapter also provides an overview of recommendations for the use of soil and soil amendments alongside the potential risk.

Chapter 8 draws attention to the role of Good Agricultural Practices (GAPs) in preventing pathogenic microbial contamination of fresh produce. The chapter begins by describing the evolution of GAPs from voluntary to regulation, then moves on to highlight the reasons for adopting these GAPs in fresh produce safety. A section on why GAPs remain the foundation for fresh produce is also provided, followed by an overview of commitment and training involved in the implementation of GAPs. The chapter reviews the scalability of GAPs and the feasibility of implementing them. A section on ways of assessing risks is also included, focusing specifically on practices such as worker training, preharvest, harvest and postharvest water, soil amendments, wildlife and domesticated animals, sanitation and storage and transportation. The chapter moves on to examine how GAPs can be improved, how produce safety can add to farming stress, as well as the relevance of GAPs for the industry in the future.

The subject of Chapter 9 is advances in sanitising techniques and their assessment for assuring the safety of fresh produce. The chapter first reviews the importance of postharvest decontamination, drawing attention to the limitations of postharvest washing. This is then followed by an analysis of the advances in postharvest washing processes such as monitoring chlorine concentration, predicting chlorine demand of wash waters, pre-oxidation of wash water and advances in alternative sanitisers. The chapter moves on to review non-aqueous decontamination methods such as electron beams, ultraviolet light, gas-phase decontamination, application of gas plasma and the gas-phase hydroxyl-radical process. Considerations for developing a

standard validation method for fresh produce decontamination methods are also reviewed.

Chapter 10 draws specific attention to developments in packaging techniques and their assessment for assuring the safety of fresh produce. The chapter begins by describing the current packaging methods in use for physical protection. It moves on to analyse the use of modified atmosphere packaging, focusing on the history and scope of the modified atmosphere packing concept, highlights the different types and packaging materials used, how a modified atmosphere is created and also the use of modified humidity packaging. A section on the use of edible coatings and films is also provided, followed by a section on active packaging methods.

The next chapter focuses on the role of good manufacturing practice (GMP) and hazard analysis and critical control point (HACCP) systems in maintaining the safety of minimally-processed fresh produce. Chapter 11 first examines the key hazards in minimally-processed fresh produce and lessons from previous outbreaks and incidents. It then goes on to review the developments in GMPs for application in minimally-processed fresh produce. A section on the application of HACCP system in minimally-processed fresh produce is also included. The chapter also considers current GMP and HACCP system practices to assure the safety of minimally-processed fresh produce.

The final chapter of the book focuses on improving safe consumer handling of fresh produce. Chapter 12 begins by first discussing consumer produce consumption, drawing specific attention to the consumption of high risk produce items. The chapter then moves on to review pathogens isolated from produce samples, which is followed by an analysis of outbreaks and illnesses caused by produce. A section on organic produce is also provided, focusing specifically on microbial quality of conventionally grown versus organic produce and foodborne outbreaks associated with organic foods. The chapter also provides an overview of farmers markets, comparing the quality of produce items from these markets to traditional retail and also identifies the foodborne illnesses that can occur due to farmers markets. Consumer knowledge and behaviours around safe produce handling and consumption is also discussed, followed by an overview of produce safety and vulnerable and immunocompromised consumers. The chapter includes an overview of fresh produce safety handling recommendations and resources, as well as a discussion on the need to include behaviour theories and formative research in the development of new and targeted education materials around produce safety.

Chapter 1

Advances in understanding contamination of fresh produce by *Salmonella*

Shirley A. Micallef, University of Maryland, USA

- 1 Introduction
- 2 The diversity of the *Salmonella*-plant association
- 3 *Salmonella* contamination of fruit and vegetables
- 4 The metabolite landscape in the plant niche
- 5 *Salmonella* on produce
- 6 *Salmonella* strategies in the plant niche
- 7 Conclusion
- 8 References

1 Introduction

Salmonella enterica is a member of the Enterobacteriaceae, a bacterial family that was last emended in 2016 based on genome- and multi-gene-based phylogenetic trees (Adeolu et al., 2016). The family contains other taxa of importance to public health (e.g. *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Shigella* and *Citrobacter*). *S. enterica* is accepted as the type species of the genus *Salmonella* and comprises six subspecies, *S. enterica* subsp. *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizona* (IIIb), *houtenae* (IV) and *indica* (VI) (Tindall et al., 2005). Food safety is primarily concerned with *S. enterica* subsp. *enterica* which is subtyped based on surface antigens yielding over 1500 serovars (Grimont and Weill, 2007) and can be divided into typhoidal (e.g. *S. enterica* serovar Typhi) and non-typhoidal (e.g. *S. enterica* serovar Typhimurium) types. Further strain differentiation within serovars has been resolved by DNA fingerprinting methods such as pulsed-field gel electrophoresis (PFPG) and multiple-locus variable numbers of tandem repeats analysis (MLVA), the most widely adopted techniques in source tracking before the advent of whole genome sequencing (WGS) (Tang et al., 2019). Typhoidal *Salmonella* has declined remarkably in many parts of the world although it continues to be a burden in some countries in Asia, Africa and Oceania (Stanaway et al., 2019). On the other hand, food safety concerns with non-typhoidal salmonellae,

which exhibit an astounding capacity to persist in various foods and in food production areas, constitute a primary concern on a global scale (Majowicz et al., 2010).

Non-typhoidal *S. enterica* subsp. *enterica* (hereafter referred to as *Salmonella*) is a diverse and adaptable taxon. Although best studied for its colonization and infection of vertebrate gastrointestinal tracts, *Salmonella* also colonizes insects such as flies and cockroaches (Nasirian, 2019; Wales et al., 2010) and can infect *Caenorhabditis elegans* (Aballay et al., 2000). Its implication in foodborne illness outbreaks involving fruit and vegetables also points to the ability of *Salmonella* to associate with plants (Callejón et al., 2015), supporting the notion that plants can serve as transitional hosts for this pathogen before returning to an enteric lifestyle, following ingestion of the colonised plant material by herbivores (Fletcher et al., 2013). In addition, *Salmonella* can persist in various environments including surface water, reclaimed wastewater, sediments and manure-amended soil (Callahan et al., 2019; Haley et al., 2009; Pornsukarom and Thakur, 2016; Sharma et al., 2020; Walters et al., 2013). Surveys of produce production areas where multiple samples types were collected for bacterial isolation suggest that surface water may present a more favourable habitat for *Salmonella* than soil (Bell et al., 2015; Gorski et al., 2011; Micallef et al., 2012). Mammals, birds, amphibians and reptiles are likely sources of environmental *Salmonella* as related strains have been recovered from wildlife and environmental samples (Gorski et al., 2011; Gruszynski et al., 2014). Once introduced in an environmental medium, *Salmonella* populations may be able to persist for prolonged periods independently from recurrent episodes of introduction (Čučak et al., 2018; Topalcengiz et al., 2020). Moreover, several studies have reported the co-existence of multiple *Salmonella* serovars (Callahan et al., 2019; Gorski et al., 2022), demonstrating the high diversity of *Salmonella* in the agro-environment, which probably increases the likelihood of contamination events. The risk of contamination of plant-based foods during production is linked to this diverse genetic pool in the environment, enhancing the probability of possessing traits that give various *Salmonella* strains the genetic mechanisms needed to successfully associate with plants.

2 The diversity of the *Salmonella*-plant association

Salmonellosis outbreaks have been linked to an impressive diversity of vegetable, fruit, nut, herb and spice crops. Examples of plant-based foods that have been contaminated with *Salmonella* leading to illnesses are given here and have included edible parts of plant members of the Solanaceae (tomatoes, peppers and paprika) (Barton Behravesh et al., 2011; Bennett et al., 2015; Lehmacher et al., 1995), the Cucurbitaceae (melons and cucumber) (Angelo et al., 2015; Laughlin et al., 2019; Walsh et al., 2014), the Rosaceae (nuts such

as almonds and stone fruit such as peaches) (CDC, 2020; Isaacs et al., 2005), the Fabaceae (alfalfa sprouts and peanuts) (CDC, 2009; Harfield et al., 2019; Kirk et al., 2004; Sheth et al., 2011), the Asteraceae (lettuce) (Lienemann et al., 2011), the Brassicaceae (arugula/rocket) (Nygård et al., 2008), the Caricaceae (papaya) (Hassan et al., 2019), the Alliaceae (onions) (McCormic et al., 2022), the Apiaceae (herbs such as cilantro and parsley) (Campbell et al., 2001), the Anacardiaceae (mango and pistachio nuts) (CDC, 2016, 2012) and others.

The United States Interagency Food Safety Analytics Collaboration (IFSAC) publishes source attribution estimates for human salmonellosis infections from all foods that occurred in the United States between 1998 and 2019 (IFSAC, 2021). *Salmonella* was the cause of 61.6% of outbreaks from all foods, based on a method used to assess outbreaks caused by *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp., with additional weight given to outbreaks occurring in the most recent 5 years (IFSAC, 2021). IFSAC categorizes plant-based foods as vegetable row crops (leafy greens), seeded vegetables (tomatoes, cucumber, pepper), fruit, other produce (such as nuts), sprouts and grains/beans (IFSAC, 2021). Plant-based foods accounted for 42.7% of all *Salmonella* illnesses from all foods, with 13.5% of infections attributed to fruit, 12.6% to seeded vegetables, 7.3% to other produce, 4.2% to vegetable row crops, 4.2% to sprouts and 0.9% to grains and beans. Fruit was ranked the second-highest category for a number of cases after chicken, and seeded vegetables and other produce were ranked fourth and fifth, respectively, with the top five categories making up 63% of all illnesses. Despite proactive measures to minimize food safety problems through grower and industry guidance and government regulations, *Salmonella* in fresh produce remains a concern of the highest priority for food safety.

Certain serovars are repeatedly associated with produce commodities (Jackson et al., 2013). Using the IFSAC categorization and according to the United States National Outbreak Reporting System (NORS) data for outbreaks occurring between 1999 and 2000, the serovars associated with the most outbreaks from plant-based food were primarily *Salmonella* Newport, followed by *Salmonella* Javiana, Enteritidis, Typhimurium and Saintpaul (CDC, 2022). *Salmonella* Newport accounted for 33% of seeded vegetable outbreaks, 25% of underground-grown vegetable outbreaks, 18% of herb and 16% of fruit-associated outbreaks. *Salmonella* Javiana is responsible for several outbreaks linked to vegetables grown underground and *Salmonella* Enteritidis caused 26% of row crop, 21% of sprout, 18% of herb and 14% of seed/nut outbreaks (CDC, 2022). These serovars may be considered generalists as they also cause several meat, poultry, dairy or egg-related outbreaks. However, some serovars may recur with the same commodity, such that serovar-commodity pairs may not only be a factor of the geographical prevalence of a particular serovar but also a consequence of serovar and commodity characteristics that make the

interaction particularly favourable. Examples of these recurring associations include *Salmonella* Poona on cantaloupe melons and more recently cucumber (Laughlin et al., 2019; Walsh et al., 2014), and *Salmonella* Newport on tomato and more recently cucumber and onion (Angelo et al., 2015; Bennett et al., 2015; Greene et al., 2008; McCormic et al., 2022). Other serovars less specifically associated with one commodity but frequently involved in fresh produce contamination issues include *Salmonella* serovars Saintpaul, Braenderup, Enteritidis and Javiana (Barton Behravesh et al., 2011; Bennett et al., 2015; CDC, 2020; Isaacs et al., 2005). Sprouts are one exception to this idea of specific pathogen-commodity pairs, as the multitude of sprout outbreaks that have been reported in the last decades have involved an astounding range of *Salmonella* serovars (Miyahira and Antunes, 2021), placing sprouts in a food safety category of their own.

3 *Salmonella* contamination of fruit and vegetables

Since *Salmonella* is prevalent in the environment, multiple routes of transmission of *Salmonella* to fresh produce crops have been identified. Water is of particular concern as it can act as a reservoir and a vehicle for the dissemination of *Salmonella* to crops. Moreover, water is used in multiple processes in both the pre- and post-harvest settings, providing many opportunities for *Salmonella* to come in contact with fresh produce during production, harvest and post-harvest handling of fruit, vegetables and nuts. During production, water is used primarily for irrigation. However, many other practices make use of copious amounts of water that come directly in contact with the crop, such as pesticide mixing and application, frost protection and evapotranspiration for cooling harvests. Experimental evidence linking agricultural practices to *Salmonella* dissemination to crops points to irrigation and rain splash as factors that increase pathogen transmission risk in the field (Cevallos-Cevallos et al., 2012; Gu et al., 2018). Other identified risk factors have implicated soil amendment use, mulching and wildlife (Cevallos-Cevallos et al., 2012; Jay-Russell, 2013; Strawn et al., 2013).

Despite the potential widespread prevalence of *Salmonella* in the environment, tracing back an outbreak to an environmental source has proven to be challenging. A few salmonellosis outbreaks implicating crops have tentatively source-tracked contamination back to irrigation water (Barton Behravesh et al., 2011; Greene et al., 2008; Voelker, 2021), and *Salmonella* isolates recovered from surface water have been reported to match illness outbreak strains in some cases (Li et al., 2014). However, most outbreak investigations fail to establish an environmental source for the contamination, even when the production area or facility is identified. This could be due to the sporadic and ephemeral nature of *Salmonella* presence in the agro-environment. Its detection could be restricted

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