



# Fresh vegetables and fruit as a source of *Salmonella* bacteria

Beata Kowalska<sup>1,A-F</sup>  

<sup>1</sup> The National Institute of Horticultural Research, Skierniewice, Poland

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## Abstract

**Introduction.** Increased consumer awareness of the health aspects of the diet has influenced the increased consumption of fruit and vegetables. Due to the fact that these products are mainly consumed raw and are usually not subjected to processes that reduce their microbiological contamination, they become a source of infection and transmit pathogens causing food poisoning in humans. *Salmonella* bacteria are a serious treat to human health and remain a serious problem in many parts of the world.

**Objective.** The aim of this study was to review the current state of knowledge regarding the prevalence of *Salmonella* bacteria on fresh fruit and vegetables. Attention is also given to the mechanisms by which these bacteria adapt to colonize plants. Methods that can prevent contamination of plant products by the bacteria are also analyzed.

**Review Methods.** The review was based on data obtained from scientific articles published in the Science Direct and Pub Med database between 2007–2022, found with the use of the following keywords: *Salmonella*, fruit, vegetables, food contamination.

**Brief description of the state of knowledge.** Data from the literature report that fresh fruit and vegetables are a source of *Salmonella* contamination through contact with soil, manure, compost, water or staff.

**Summary.** Actions targeting salmonellosis prevention should be undertaken by both the public and private sectors. Government regulations and stricter measures put in place can provide a framework that guides both domestic production and international imports. Periodic training of workers dealing with food is also important. Attention should be directed mainly to production control and less to the testing of final products. Education leading to increased awareness of salmonellosis should be indispensable.

## Key words

food safety, vegetables, fruit, *Salmonella*, salmonellosis, food contamination

## INTRODUCTION AND OBJECTIVE

Increased consumer awareness of the health aspects of the diet has influenced an increased consumption of vegetables and fruit [1, 2]. Due to the fact that these products are mainly consumed raw and are usually not subjected to processes that reduce their microbiological contamination, they become a source of infection and transmit pathogens causing food poisoning in humans. Notable among the bacteria posing a particular epidemiological threat to humans are *Salmonella*, characterised by high levels of pathogenicity and a wide range of hosts. Despite global improvements in public health protection, salmonellosis remains a serious problem in many parts of the world [3, 4, 5, 6].

The aim of this study was to review the current state of knowledge regarding the prevalence of *Salmonella* bacteria on fresh vegetables and fruit in the context of possible food poisoning. It will also highlight the mechanisms by which these bacteria adapt to colonize plants. Sources of contamination of plant products by these bacteria are also discussed.

## REVIEW METHODS

The review was based on data obtained from scientific articles published in the Science Direct and Pub Med database between 2007–2022, found with the use of the following keywords: *Salmonella*, fruit, vegetables, food contamination.

## DESCRIPTION OF THE STATE OF KNOWLEDGE

Bacteria of the genus *Salmonella* are Gram-negative, 0.7–1.5 × 2–5 µm in size which move by means of flagella. The genus *Salmonella* are rod-shaped, belonging to the family Enterobacteriaceae. They have several endotoxins: antigens O, H and Vi, and belong to the facultative anaerobes, chemoorganotrophs, carrying out aerobic metabolism and fermentation processes. The optimum temperature for their growth is 37 °C, but they grow over a wide temperature range from 2 °C – 54 °C. These bacteria are oxidase-negative and catalase-positive, producing hydrogen sulphide and do not hydrolyse urea. They grow on KCN medium (Moeller's medium), which is a selective medium used to check whether the microorganism shows growth in the presence of potassium cyanide [7].

Bacteria of the genus *Salmonella* mainly cause poisoning of the gastrointestinal tract and the infections they cause are referred to as salmonellosis, the symptoms of which usually are: headache, abdominal pain, diarrhoea and vomiting,

Address for correspondence: Beata Kowalska, Instytut Ogródnictwa Państwowy Instytut Badawczy, Skierniewice, Poland  
E-mail: beata.kowalska@inhort.pl

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occurring 6 – 8 hours after the pathogens enter the body. Those most at risk of acute infection are children under one year of age and adults over 60 years of age. Infection occurs via the oral route. In the vast majority of cases, the source of infection is the ingestion of contaminated food. For many years, salmonellosis has been the most common cause of bacterial food poisoning worldwide [5, 6].

The genus *Salmonella* is divided into two species, *S. bongori* and *S. enterica*. Additionally, the species *S. enterica* is divided into seven subspecies, of which *S. enterica* subsp. *enterica* is the main causative agent of salmonellosis in humans. However, the most commonly isolated serogroup from humans is *S. enteritidis*, the second most commonly isolated serogroup is *S. typhimurium* and the third – *S. infantis* [7].

**Occurrence of *Salmonella* on vegetables.** Literature data report that fresh vegetables can be contaminated with *Salmonella* bacteria [1, 8, 9, 10, 11, 12, 13, 14, 15, 16]. Particularly hazardous to human health are those eaten raw, without heat treatment and only washed under running water. Sher et al. [6] analyzed the sources of outbreaks caused by *Salmonella* bacteria in the United States between 1990 – 2015, and found that of 1,200 outbreaks, 96 (8%) were due to the consumption of contaminated vegetables. Leafy vegetables, which have a large surface area that can be colonized by *Salmonella*, are a particularly serious risk [17]. These vegetables are grown on a large scale, both in the field and under roof, and are exported fresh as well as minimally processed. Azimirad et al. [8] investigated the contamination of leafy vegetables, i.e. watercress, leek, basil, savory, parsley, radish. They detected *S. enterica* subsp. *enterica* in 15/274 (5.5%) of samples of leafy vegetables – unwashed and unpackaged, and in 4/93 (4.3%) samples of ready-to-eat, washed, cut and packaged (RTE) leafy vegetables. These studies suggest that *Salmonella* contamination can occur at all stages of vegetable production, not only in the field during cultivation and harvesting, but also during washing, cutting, packaging and transportation.

Lettuce (*Lactuca sativa* L.), one of the most consumed leafy vegetables in the world, is often a carrier of *Salmonella* spp. and *Escherichia coli* O157:H7 bacteria. Researchers from Brazil analyzed 1,296 articles on *Salmonella* colonization of lettuce [18]. The study was conducted using statistical methods – meta analysis, and found that the average prevalence of *Salmonella* spp. on lettuce was 0.041, with a wide range from 0.001 for Japan to 0.5 for Burkina Faso, West Africa. For developed countries, the value averages 0.028 and for developing countries 0.064. A rather high variation between regions or countries was also observed, for example, a higher prevalence was found for Europe – 0.135, than for the United States and Canada – 0.002, while Central and South America, Asia and Africa have similar prevalence rates of 0.036, 0.063 and 0.079, respectively. The density of *Salmonella* bacteria on lettuce leaves was also studied. This value, expressed as the most likely number per gram, ranges from 0.054–218.78 [18].

A study of vegetables, mainly lettuce, sold at a market in Vietnam reported that 12.9% of vegetable samples were contaminated by *Salmonella* bacteria. It was additionally observed that the number of infected vegetables was higher in the rainy season than in the dry season [13]. In Canada, *Salmonella* bacteria were detected in 18/6032 (0.29%) of leafy herb samples [9]. Despite the low prevalence of *Salmonella* spp. on lettuce, the widespread consumption of this vegetable

carries a risk of poisoning, mainly due to the lack of heat treatment before consumption. Green leafy vegetables have the highest priority for fresh produce safety in a global perspective. The application of Good Agricultural Practices (GAPs) is widely recognized as the most significant measure in reducing *Salmonella* and other foodborne pathogens in fresh produce. GAPs are guiding principles focusing on best agricultural practices in fresh produce production, cultivation, packaging, handling, storage and transportation [19].

Researchers in Malaysia tested fresh vegetables purchased from supermarkets and markets for *Salmonella* [16]. They analyzed head cabbage, lettuce, cucumbers, tomatoes and carrots. The presence of *Salmonella* was found in 3.3% of supermarket cabbage samples, with densities ranging from <3.0 NPL g<sup>-1</sup> to >15 NPL g<sup>-1</sup>. *S. enterica* serovar Typhimurium bacteria were detected in 10% of cucumber samples purchased from supermarkets and in 20% of samples from a market, with a density ranging from 3.0 NPL g<sup>-1</sup> – 1,100 NPL g<sup>-1</sup>. These data indicate that cabbage and cucumber pose a serious risk of salmonellosis in Malaysia [16].

Tomatoes can also be colonised by *Salmonella* bacteria. Contamination and internalisation of bacterial cells with tomato tissues can occur through contaminated water used for watering or spraying the plants. Studies indicate that contaminated soil leads to contamination of plant tissues, including fruit. *Salmonella* cells can enter the plant through natural openings – the stomata or hydattodes. In addition, trichomes and internal transport from the leaves through the phloem is indicated as a transport route of bacterial cells to the fruit.

In a study in Spain [14], *Salmonella* bacteria were also found to survive on the surface of cucumbers after artificial inoculation. Their abundance was tested several times over a 24-hour period. It was found that within the first two hours after infection, the bacterial count decreased 10-fold, increased over the next several hours, and reached the initial count after 24 hours. It was also observed that *Salmonella* bacteria are much more resistant to the low humidity of the environment prevailing on the cucumber fruit surface than *E. coli* bacteria, the number of which dropped dramatically as early as eight hours after infection. These studies confirm the fact that *Salmonella* bacteria survive on the surface of cucumbers during storage at room temperature. In order to protect consumers from microbial contamination, proper sanitation must be maintained during cultivation, harvesting and distribution, i.e. throughout the whole production chain [14].

**Occurrence of *Salmonella* on fruit.** *Salmonella* can also occur on fruit and contaminate the produce [1, 12, 20, 21]. Strawberries, due to the fact that they are in direct contact with the soil, are particularly vulnerable to *Salmonella* infection [22, 23, 24, 25]. Under experimental conditions, the fungi *Botrytis cinerea* and *Rhizopus stolonifer* present on the surface of strawberries, were found to create unfavourable conditions for *Salmonella enterica*. On the other hand, it has been proven that damage caused by plant pathogens increases the penetration of pathogenic bacteria into plant tissues. Bacteria that inhabit the inner tissues of plants are much more difficult to eliminate than those that reside on the plant surface.

The incidence of *Salmonella* on fruit depends not only on the conditions of primary production of the raw materials,

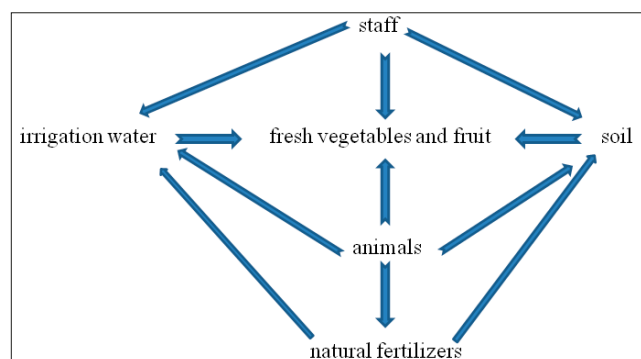
but also during processing and packaging. In Spain, 152 samples of fresh strawberries and 31 samples of frozen strawberries were tested. *Salmonella* was not detected in any of the samples [24].

Contamination of fruit produce with bacterial pathogens such as *Salmonella* usually occurs sporadically, however, this is still unacceptable as it represents a food safety risk.

**Factors affecting *Salmonella* contamination of vegetables and fruit.** The significant increase in the number of *Salmonella* infections in humans after eating raw fruit and vegetables recorded in recent years is due to the following factors:

- agro-technical mistakes made during cultivation, including the use of improperly prepared organic fertilizers or the application of fertilizers at the wrong time [26];
- use of contaminated water to irrigate plantations [27];
- lack of proper staff hygiene during field work [1, 4, 28];
- climate change, especially high temperatures, floods, sudden events, mainly hailstorms, strong winds [29].

*Salmonella* contamination of fresh vegetables and fruit in the farm-to-fork chain is presented in Figure 1. Natural fertilizers, including manure, pig slurry, cattle and chicken manure, are excellent sources of enrichment for crop production, especially on organic farms. The improper use of organic fertilizers leads to contamination of soils, crops and, consequently, human infections. Efforts should be made to eliminate pathogenic organisms present in organic fertilizers even before they are applied to the soil [10, 30]. The presence of *Salmonella* in organic fertilizers used for plant cultivation is unacceptable [31].



**Figure 1.** *Salmonella* contamination of fresh vegetables and fruit in the farm-to-fork chain. The arrows are presenting the direction of *Salmonella* transfer in the environment.

Reports from the literature indicate that the non-use of chemical pesticides in organic crops may create a susceptible environment for the development of pathological disorders in organic fruit and vegetables [32]. Additionally, in these crops, pests easily damage the skin of the fruit, creating entry points for pathogenic microorganisms into the fruit [20].

A common source of infection is the water used for watering, irrigation, spraying or washing. Water is most often contaminated through improperly discharged wastewater, uncleaned reservoirs, leaks from reservoirs or manure storage areas, livestock and wildlife faeces, or run-off water from contaminated fields. Water used for spraying and washing fruit and vegetables should meet drinking water standards [25, 27]. The potential infection of plants with waterborne pathogenic bacteria is increasing in developing countries,

as wastewater is often used for watering in these countries. Studies report that a single application of *Salmonella*-infected water to carrots and radishes resulted in contamination of these vegetables detected at harvest [10]. A similar phenomenon was observed with spinach watered with water artificially contaminated with *Salmonella* at 7.12 log cfu ml<sup>-1</sup>. *Salmonella* bacteria were detected in leaves, stems and roots of spinach at the following rates, respectively: 3.56; 3.04; 4.03 log cfu ml<sup>-1</sup> of macerated tissue solution [33]. Watering *Salmonella*-contaminated strawberries during the growing season resulted in severe fruit infection; it was noted that drip irrigation is a more hygienic and safer method than irrigation by sprinkling [22, 25].

The risk of contamination of vegetables by pathogenic bacteria increases in hydroponic crops, where there is direct contact between water and the edible parts of the vegetables [34]. The degree of internalisation of fresh produce, including leafy vegetables, mainly depends on the size of the inoculum in the culture medium. The survival and growth of *S. enteritidis* on the plant surface is also strongly influenced by pH and temperature. Under conditions of reduced pH and temperature around 21 °C, the growth rate of *Salmonella* is lower compared to optimal conditions. The age of the plant influences the *Salmonella* bacteria colonization of lettuce leaves. Small plants were more susceptible to colonization than older plants. The presence of *S. enteritidis* in hydroponic media and their internalisation led to an increase in the indices responsible for defence – H<sub>2</sub>O<sub>2</sub> and malondialdehyde (MDA), and enhanced the defence mechanisms, i.e. production of phenols and antioxidants [34].

**Survival of *Salmonella* bacteria in soil.** Literature data report that *Salmonella* have a high degree of resistance to adverse conditions and are able to survive in soil for long periods of time, up to 500 days [35]. The survival period of bacteria in the soil environment is influenced by a number of factors: temperature, pH and ambient humidity, nutrient availability, soil type, bacterial serotype, as well as the presence of bacteriophages or antagonistic microorganisms. For example, *S. enteritidis* was found to survive in clayey sand during summer for 74 days, while it survives for 186 days in autumn in loess soil. In manure-enriched soil, *Salmonella* bacteria are able to survive for up to 332 days [36]. Jechalke et al. [37] demonstrated that the use of high-quality manures (e.g. containing a high organic matter content) can reduce *Salmonella* survival in soil. High nutrient levels can increase the activity and biodiversity of soil microorganisms, including those antagonistic to *Salmonella* [38]. *Salmonella* introduced into the soil with contaminated water were detected 203 days after application [10]. In a study by Hruby et al. [26], *Salmonella* were detected in soil one year after introduction with chicken manure.

The risk of contamination of plants in the field also depends on the concentration of the pathogen in the soil and the distance of the soil to the edible parts of the plant. One of the main routes of *Salmonella* transfer to the edible parts of the plant is through seeds germinating in contaminated soil enriched with manure of zoonotic origin. The roots and above ground parts of cress and oat seedlings were found to be colonized by *S. typhimurium* after their seeds had germinated in artificially contaminated soil. It was also observed that the density of bacterial cells on roots and leaves was comparable [26].

Soil type influences the survival of *Salmonella* in an agro-environment. Survival of *Salmonella* in clay soil was higher than in sandy soil; this was related to both higher nitrogen and organic carbon content and higher microbial biodiversity – high biodiversity of indigenous soil microorganisms results in lower *Salmonella* abundance [38].

One source of pathogenic microorganisms, including but not limited to *Salmonella*, is wastewater discharged into surface waters. The presence of *Salmonella* in inadequately treated wastewater poses a risk of contamination of the environment, especially water and soils followed by vegetables and fruit [21].

Environmental conditions have a great influence on the survival of *Salmonella* on plant tissues. In a study conducted in Canada on more than 31,000 samples of fruit and vegetables (leafy herbs, leafy vegetables, onions, melons, tomatoes and berries), microbial contamination of the products tested, especially leafy vegetables, was found to be highly dependent on the season, with greater bacterial contamination occurring in samples taken during the summer. In addition, greater contamination of vegetables from organic farms was noted compared to those from integrated farms [9].

Significant climate change has been observed for many years, including global warming, changes in precipitation patterns, and extreme weather events. These anomalies have a huge impact on the survival of pathogenic bacteria, including *Salmonella*, in soil environments and in/on plants [29]. Ge et al. [39] report that under extreme weather conditions, with a high inoculum of *Salmonella* in the soil (8–9 log cfu g<sup>-1</sup> soil), the level of internalisation of lettuce leaves with the bacteria was significantly higher than under normal weather conditions. Under drought conditions, the levels of lettuce leaf contamination increased 16-fold with an inoculum of 8 log cfu g<sup>-1</sup> soil and 27-fold with an inoculum of 9 log cfu g<sup>-1</sup> soil. Excess water in the soil can block the transport of oxygen and other gases between the roots and the atmosphere; consequently, the lack of oxygen leads to the aggregation of minerals around the roots, which contribute to damage on the plants. This damage becomes an easy route for pathogens to enter the plant, which are then transported to the edible parts of the plant [39].

#### Colonisation mechanism of plants by *Salmonella*.

*Salmonella* bacteria are not common in agricultural environments. If they colonize plant surfaces, they are treated as microbial contaminants. *Salmonella* bacteria also colonize internal plant tissues as endophytes [11, 40], penetrating through wounds, stomata, hydrotodes and trichomes. Colonization of the plant interior is enabled by the following characteristics of *Salmonella*: mobility, chemotaxis capacity, presence of a type III secretion system (T3SS), and biofilm formation [11, 41]. These bacteria not only colonize internal plant tissues, but also have the ability to multiply inside the plant in intercellular spaces. Under experimental conditions the ability of *Salmonella* to colonize external and internal plant tissues was found to depend not only on the bacterial strain, but also on the type of host [11]. For example, the isolate *S. enterica* serovar Typhimurium S1 readily colonized tomato, lettuce and celery roots, while it colonized spinach roots to a much lesser extent. Endophytic colonization of tomato and celery tissues was also higher than that of spinach tissues. In the case of another isolate of *S. enterica* serovar Typhimurium, the highest number of bacteria was found on

the surface of lettuce and maize, a lower number on spinach, tomato and carrot, while the surface of parsley and celery was the least colonized [11].

Three mechanisms are presented in the literature that enable *Salmonella* bacteria to colonize plant tissues: adaptation, avoidance, suppression [42]. Preadaptation to mild stress conditions enhances the survival of the pathogen under more severe conditions. Such adaptation can be achieved when *Salmonella* survives in soil enriched in organic material before colonizing the plant. It has been observed that exposure of the pathogen to drought stress leads to tolerance of *S. enterica* to other stress factors, e.g. high temperature, disinfectants or UV light.

The avoidance strategy is to inhibit the induction of the immune system of colonized plants. Bacteria of the genus *Salmonella* contain several virulent factors on the genome. Among them are factors related to mobility and adhesion, such as flagella, O-antigen, fimbriae and cellulose. Type 1 fimbriae, located on the bacterial cell surface, are considered the main adhesion factor [42]. Adhesion plays an important role in pathogenesis processes, enabling pathogenic microorganisms to colonize specific ecological niches and protecting them from the action of non-specific host defence mechanisms. The adhesion process is a reaction between a bacterial ligand (adhesin) and a complementary receptor located on the surface of a eukaryotic cell.

*Salmonella* bacteria have evolved a mechanism involved in the process of evasion of recognition by plant receptors. Virulence genes clustered in regions on the chromosome, called *Salmonella* pathogenicity islands (SPIs), play an important role in the suppressive strategy. Five major pathogenicity islands (SPIs 1–5) have been identified [42].

*Salmonella* bacteria have the ability to form a biofilm – a compact bacterial complex, a layer that is difficult to remove from surfaces. The formation of a bacterial biofilm matrix is a complex and multi-step process. Regardless of the type of substrate on which the biofilm is formed, the first stage is primary adhesion determined by electrostatic attraction, during which a contact layer is formed. At this stage, fimbriae play an important role. During the next stage, the production of extracellular matrix EPS (Extracellular Polymeric Substances) and the formation of the core layer of the biofilm irreversibly bound to the substrate begin. Irreversible adhesion is associated with the proliferation of bacterial cells and the activation of their internal communication process called quorum sensing (QS), leading to the formation of a mature biofilm [10, 43].

Due to the strong attachment of adhering pathogens to the surface of products, facilitated by surface irregularities, i.e. roughness, crevices, indentations, washing does little to reduce their numbers. In a study comparing the surface roughness of basil, lemon basil, pepper and cabbage leaves, it was found that the adhesion capacity of *Salmonella* cells on the leaf surface increased with leaf roughness. Pepper leaves showed the highest roughness and thus were colonized by the highest number of cells compared to the other vegetables tested. This was followed by lemon basil, basil and cabbage. The genus *Salmonella*, despite exposure to environmental stress, successfully persisted on the plant surface. This is also related to its production of cellulose and other polysaccharides, its ability to penetrate plant tissue, and its ability to incorporate into already existing multispecies biofilms [17].

**The problem of antibiotic resistance.** Antibiotic resistance has been recognized as a global health problem and as one of the most important health challenges of the 21st century. The emergence of antimicrobial-resistant bacteria in food, including fresh produce, has become a challenge and a major public health problem worldwide. The widespread use of antimicrobials in agriculture exposes people to antibiotic-resistant bacteria. Antibiotic-resistant bacteria have been identified in animal waste, sewage, river sediment and soil. The consumption of fresh produce, especially raw produce, provides a route for direct human exposure to resistant microorganisms. Antibiotic-resistant *Salmonella* towards vancomycin, erythromycin, ampicillin and penicillin, have been isolated from vegetables [44, 45].

## SUMMARY

Fresh plant materials are subjected to various processes in order to prepare a minimally processed product that will be valuable and attractive to the consumer. Such processes include washing, cleaning, removal of plant parts, cutting, etc. It is important to be aware that washing plants only removes microorganisms from their surfaces to a small extent. No other technology, apart from heat treatment and radiation fixation of food, can eliminate human pathogens. Therefore, preventive treatments are essential, paying attention to good practices in field production and processing [46].

The maintenance of proper hygiene rules by personnel involved in the care and harvesting of plants is also an important issue. Leafy vegetables, due to their nature, are harvested by hand; therefore, the maintenance of Good Agricultural Practices (GAP) and Good Handling Practices (GHP) during harvesting with respect to personal hygiene, the use of protective gloves and the disinfection of tools, are crucial for avoiding the microbial contamination of horticultural products which are consumed raw [15]. Reducing the spread of *Salmonella* requires control measures that start at the production stage of fruit and vegetables in the field, and end when these products reach the table of consumers – a process which must be part of the farm-to-table strategy. In 2018, the WHO issued recommendations relating to *Salmonella* control throughout the food production chain. These efforts aim to strengthen food safety standards that enhance effective surveillance, educate consumers and train food handlers on best practices in preventing salmonellosis [47].

Actions targeting salmonellosis prevention should be undertaken by both the public and private sectors. Government regulations and stricter measures put in place can provide a framework that guides both domestic production and international imports. Periodic training of workers dealing with food is also important. Attention should be directed more to production control and less to the testing of final products. Education of the public leading to increased awareness of salmonellosis and potential sources of *Salmonella* infection should become an indispensable element.

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