Journal of Food Protection, Vol. 79, No. 1, 2016, Pages 6–16
doi:10.4315/0362-028X.JFP-15-274

Prevalence and Characteristics of *Salmonella* Serotypes Isolated from Fresh Produce Marketed in the United States

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MS 15-274: Received 30 June 2015/Accepted 27 August 2015

ABSTRACT

Salmonella continues to rank as one of the most costly foodborne pathogens, and more illnesses are now associated with the consumption of fresh produce. The U.S. Department of Agriculture Microbiological Data Program (MDP) sampled select commodities of fresh fruit and vegetables and tested them for *Salmonella,* pathogenic *Escherichia coli,* and *Listeria*. The *Salmonella* strains isolated were further characterized by serotype, antimicrobial resistance, and pulsed-field gel electrophoresis profile. This article summarizes the *Salmonella* data collected by the MDP between 2002 and 2012. The results show that the rates of *Salmonella* prevalence ranged from absent to 0.34% in cilantro. A total of 152 isolates consisting of over 50 different serotypes were isolated from the various produce types, and the top five were *Salmonella enterica* serotype Cubana, *S. enterica* subspecies *arizonae* (subsp. IIIa) and *diarizonae* (subsp. IIIb), and *S. enterica* serotypes Newport, Javiana, and Infantis. Among these, *Salmonella* serotypes Newport and Javiana are also listed among the top five *Salmonella* serotypes that caused most foodborne outbreaks. Other serotypes that are frequent causes of infection, such as *S. enterica* serotypes Typhimurium and Enteritidis, were also found in fresh produce but were not prevalent. About 25% of the MDP samples were imported produce, including 65% of green onions, 44% of tomatoes, 42% of hot peppers, and 41% of cantaloupes. However, imported produce did not show higher numbers of *Salmonella*-positive samples, and in some products, like cilantro, all of the *Salmonella* isolates were from domestic samples. About 6.5% of the *Salmonella* isolates were resistant to the antimicrobial compounds tested, but no single commodity or serotype was found to be the most common carrier of resistant strains or of resistance. The pulsed-field gel electrophoresis profiles of the produce isolates showed similarities with *Salmonella* isolates from meat samples and from outbreaks, but there were also profile diversities among the strains within some serotypes, like *Salmonella* Newport.

According to statistics on foodborne illness, *Salmonella* almost always ranks at the top in the number of cases, hospital visits, premature death, and loss of productivity *(53)*. Most *Salmonella* infections are caused by poultry products; however, a Centers for Disease Control and Prevention (CDC) study showed that various types of fresh produce have increasingly been implicated and that 46% of the illnesses can be attributed to them *(37)*. In accordance with this, a recent source attribution study estimated that fruit and vegetables were implicated in about 50% of *Salmonella* illnesses *(15)*.

The prevalence of *Salmonella* in meat and poultry products is well established, and these data have been useful for developing hazard analysis and critical control point guidelines for slaughter houses and processing plants *(8, 42, 54, 57)*. In contrast, there are only a few reports on the presence of *Salmonella* in fresh produce, and most of these were limited studies that focused on pre- and postharvest practices for processing of ready-to-eat products *(1, 3, 28, 29, 51)*. This lack of information has limited our understanding of the prevalence of *Salmonella* on the various fresh produce types, and little is known about the characteristics of *Salmonella* strains that are present in produce, such as serotypes, antimicrobial resistance, pulsed-field gel electrophoresis (PFGE) profiles, and whether their presence in produce is epidemiologically linked to outbreaks. The Microbiological Data Program (MDP) was established in 2001 and administered by the U.S. Department of Agriculture (USDA) Agricultural Marketing Service for the purpose of monitoring foodborne pathogens in fresh produce consumed in the United States. Over a period of 11 years, from 2002 to 2012 when the program was defunded, the MDP collected an average of 12,000 fresh produce samples annually from distribution centers across the United States and tested them for the presence of *Salmonella*, enterotoxigenic *Escherichia coli* (ETEC), Shiga toxin–producing *E. coli* (STEC), including O157:H7, and *Listeria monocytogenes*. The commodities selected for sampling were commonly consumed and frequently implicated in outbreaks as determined by the statistics compiled by the CDC. The statistical framework developed by the MDP for national-level sampling, program operations, and annual reports can still be accessed at www.ams.usda.gov/mdp *(52)*. The MDP provided one of the largest publically available databases on the presence of pathogens in fresh produce, and

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TABLE 1. Salmonella *prevalence in fresh produce tested between 2001 and 2003 using the serological VIDAS assay*

Commodity	No. of samples tested	No. (%) VIDAS positive[a]	No. of *Salmonella* isolates (% of VIDAS-positive samples)[b]	% prevalence[c]
Cantaloupe	3,243	8 (0.25)	1 (12.5)	0.03
Celery	4,899	2 (0.04)	1 (50)	0.02
Lettuce	11,855	12 (0.10)	4 (33)	0.03
Tomato	7,559	10 (0.13)	1 (10)	0.01

[a] % VIDAS positive = (number of VIDAS-positive samples/total number of samples tested) × 100.
[b] % of VIDAS-positive samples = (number of confirmed isolates/number of VIDAS-positive samples) × 100.
[c] % prevalence = (number of confirmed isolates/total number of samples tested) × 100.

TABLE 2. Salmonella *prevalence in fresh produce tested between 2004 and 2012 using BAX PCR*

Commodity	No. of samples tested	No. (%) PCR positive[a]	No. of isolates (% of PCR-positive samples)[b]	% prevalence[c]
Cantaloupe	16,169	50 (0.31)	14 (28)	0.09
Celery	1,110	0	0 (0)	0
Cilantro	9,245	52 (0.56)	31 (60)	0.34
Green onions	7,332	27 (0.37)	6 (22)	0.08
Hot peppers	8,123	27 (0.33)	21 (78)	0.26
Lettuce				
Whole	10,816	39 (0.36)	7 (18)	0.06
Bagged	7,269	40 (0.4)	3 (8)	0.04
Organic	1,159	0	0 (0)	0
Total	19,244	79 (0.41)	10 (13)	0.05
Parsley	1,700	8 (0.46)	5 (63)	0.29
Spinach				
Bunch	6,926	28 (0.4)	10 (36)	0.14
Bagged	4,104	24 (0.48)	12 (50)	0.29
Total	11,030	52 (0.47)	22 (42)	0.2
Sprouts	12,976	79 (0.61)	32 (41)	0.25
Tomatoes				
Round	14,530	59 (0.41)	1 (2)	0.01
Roma	6,199	16 (0.26)	1 (6)	0.02
Cherry/grape	3,940	7 (0.18)	3 (43)	0.08
Total	24,669	82 (0.33)	5 (6)	0.02

[a] % PCR positive = (number of PCR-positive samples/total number of samples tested) × 100.
[b] % of PCR-positive samples = (number of confirmed isolates/number of PCR-positive samples) × 100.
[c] % prevalence = (number of confirmed isolates/total number of samples tested) × 100.

these data were used to determine the prevalence of STEC *(18)* and ETEC *(19)* strains in fresh produce and helped to identify produce types that are more commonly associated with these bacteria. This report summarizes the MDP survey data on *Salmonella* for the 11-year period, including prevalence rates and characterization data, such as the serotypes, antimicrobial resistance profiles, and PFGE patterns of *Salmonella* organisms isolated from various types of fresh produce.

MATERIALS AND METHODS

Fresh produce commodities. The commodities collected and tested by the MDP from 2002 to 2012 included cantaloupes, celery, cilantro, green onions, hot peppers, lettuce, parsley, spinach, alfalfa sprouts, and a variety of tomatoes. In some years, bagged ready-to-eat lettuce and spinach samples were also included. The spinach samples included both baby and mature plants, and lettuce included both romaine and iceberg, but for some of these, the numbers sampled were low and so no distinction was made as to variety. Most of the alfalfa sprouts and cherry tomato or grape tomato samples were in clam-shell packaging. Samples were collected randomly on a year-around basis from over 600 distribution centers and terminal markets located in 11 states within the continental United States. However, the broad distribution and the fluid movement of commodities from the participating distribution centers extended the product coverage to 13 additional states, including Alaska and Hawaii. The statistical framework for sampling was based on the populations of the participating states and the probability proportional to the distribution volume of the randomly selected collection sites, i.e., the amount of produce that moved through the sites. Therefore, the MDP data collected over several years may actually reflect national trends encompassing differences in geography and seasonality, in contrast to surveys that were done with smaller numbers of samples and within limited time frames *(1, 28, 29)*. The MDP samples included both domestic and imported products, and the lists of commodities collected by year, states where samples were collected, and states of origin can be found online at www.ams.usda.gov/mdp *(52)*.

Methods and characterization. Prior to 2003, the participating MDP laboratories used the VIDAS (bioMérieux, St. Louis, MO) system to serologically screen produce samples enriched in lactose broth for *Salmonella*. Beginning in 2004, the MDP implemented the BAX PCR (DuPont, Wilmington, DE) system to screen enrichment samples for *Salmonella* spp. Briefly, fresh fruit and vegetable samples were culture enriched in universal preenrichment broth. Genomic DNA from each sample was extracted and purified using the Promega Maxwell system (Promega Corporation, Madison, WI) and used as the template in a PCR assay. All PCR-positive samples were culture confirmed as follows: universal preenrichment broth cultures from which PCR-positive samples were obtained were subcultured into tetrathionate broth and Rappaport-Vassiliadis broth or SDIX RapidCheck SELECT *Salmonella* medium (Strategic Diagnostics, Inc., Newark, DE). After overnight incubation at 42°C, aliquots were plated or streaked onto several selective agar media, including brilliant green sulfa, chromogenic, xylose lactose Tergitol, xylose lysine deoxycholate, Hektoen enteric, and bismuth sulfite agars. Colonies were also tested on triple sugar iron agar, lysine iron agar, and urea agar slants. The presumptive isolates were biochemically identified using the VITEK-2 system and serologically confirmed to be *Salmonella* with the VIDAS syatem (bioMérieux). All *Salmonella* isolates were further characterized for their specific serotypes using the U.S. Food and Drug Administration *Bacteriological Analytical Manual* protocol *(55)* and tested for antimicrobial resistance and PFGE profile. Antimicrobial resistance was tested using the National Antimicrobial Resistance Monitoring System gram-negative panel and the Sensititre system (Thermo Scientific, Oakwood Village, OH). All *Salmonella* isolates were subjected to PFGE by the participating state agriculture or public health laboratories that were PFGE certified by the CDC, and the data were uploaded to the CDC PulseNet database *(40)*. Details of these procedures and annual MDP progress updates and summaries, including details of analytical testing methods used, results, and data management, can be found in the online MDP resource at www.ams.usda.gov/mdp *(52)*.

RESULTS AND DISCUSSION

***Salmonella* prevalence in produce types.** The *Salmonella* prevalence in meat and poultry ranged from 1% to 10% depending on the type of meat or produce *(8, 42, 46, 57)*. Several U.S. studies *(28, 29, 51)* showed that *Salmonella* prevalence is usually low in fresh produce (<1%), and the MDP data are consistent with these findings. In total, the microbiology laboratories of the various state agriculture departments and the USDA Agricultural Marketing Service federal facility that participated in the MDP isolated 152 *Salmonella* strains from fresh produce samples collected from 2002 to 2012. Prior to using PCR in 2004 to screen produce samples, four commodities were screened for *Salmonella* using the serological VIDAS assay. To eliminate any data variability due to differences in sensitivities and methods, the VIDAS and PCR data are presented and discussed separately. The data in Table 1 show the numbers and percentages of detection by VIDAS, and the prevalence percentage is calculated based on the numbers that were culture confirmed from the enrichment samples. All four products had many presumptive VIDAS-positive samples, but most could not be culture confirmed, so only a few *Salmonella* isolates were obtained from these, with most being from lettuce and cantaloupe (0.03%) (Table 1). Compared to the PCR data for these same commodities (Table 2), the prevalence percentage obtained with VIDAS were slightly lower. For instance, the prevalences for cantaloupes were 0.03% and 0.09% for VIDAS and PCR, respectively. These variations may be due to differences in the methods or the sensitivities and specificities of the assays *(17, 58),* but factors such as different samples, the number of samples tested, the source of the samples, and seasonality most likely all affected the results *(2)*.

Based on PCR data, the highest *Salmonella* prevalences were observed in cilantro (0.34%), parsley and spinach (0.29%), hot peppers (0.26%), and sprouts (0.25%) (Table 2). These fresh produce types have contributed to many of the *Salmonella* infections for which produce has been implicated *(15, 45)*. Parsley and cilantro have also been found to be frequent carriers of ETEC *(19),* and STEC were also commonly found in cilantro and spinach *(18)*. With STEC, however, there appeared to be a close association with spinach, as over half (70 of 132) of the STEC isolated by the MDP were from spinach *(18)*. No such link was observed for *Salmonella,* as large numbers of *Salmonella* were isolated from a variety of produce types (Table 2). Interestingly, tomatoes, which have caused a number of *Salmonella* outbreaks, were found to have low *Salmonella* prevalence (0.02%). The tomato samples tested by the MDP were retail quality and collected from distribution centers, so they had been washed and coated with mineral oil. It is possible that these postharvest handling practices may have reduced contamination levels *(52)*. Microbial surveys of fresh produce in Canada and Mexico also showed low levels of *Salmonella* contamination in tomatoes *(4, 7, 12)*. Many studies have reported that *Salmonella* can internalize in tomatoes, but the process is influenced by several factors, including the variety of tomato, *Salmonella* serotype, colonization sites on the fruit, and storage temperature *(5, 25, 60, 61)*. Internalized pathogens would be difficult to detect and may be a contributing factor to the low prevalence observed.

The prevalences of *Salmonella* calculated for the various produce types are also dependent on the effectiveness of culture confirmation. Many VIDAS-positive samples could not be confirmed, and likewise, the numbers of PCR positives ranged from 0 in celery and organic lettuce to 0.61% for sprouts (Table 2), but the actual numbers of culture-confirmed samples were much lower. The inability to culture confirm presumptive positives is not unusual, as the *Salmonella* strain may have died off by the time of confirmation, or perhaps the high levels or types of normal flora in the samples may have interfered with confirmation. With some products, however, there may be intrinsic factors that could be affecting culture confirmation. For example, tomatoes had high rates of PCR-positive results (0.33%), but culture confirmation proved to be difficult, resulting in low prevalence (0.02%). Among the tomato varieties tested, confirmation was most successful with cherry tomatoes (3 of 7, 43%) and least successful with round tomatoes (1 of 59, 2%). It is uncertain whether there may have been differences in microflora or other factors associated with each variety that interfered with the confirmation procedures.

Potential sources for pathogen contamination of produce have been identified in the field *(49)* and at preharvest *(38)* and processing *(26, 34)* stages. These included soil, manure or fertilizer application, water from irrigation or nonirrigation sources, wildlife, and processing and handling practices. Contact of the under surfaces of lower leaves with soil or contaminated irrigation water is a possible risk factor *(38)*. If it is a real one, it is likely that produce grown closer to the ground should be more susceptible to contamination. The MDP data are somewhat in agreement with this hypothesis, as products like spinach, cilantro, and parsley had the highest prevalence rates (0.29 to 0.34%). However, hot peppers (0.26%), which are grown on vines and off the ground, had *Salmonella* prevalence similar to that of spinach (0.29%), while lettuce, which is also grown in the soil, did not have high prevalence (0.05%) (Table 2). The fact that the outer leaves of lettuce are often removed and discarded during processing may have contributed to lower prevalence in this product.

Analysis of celery using the VIDAS system found 1 of ~4,900 samples tested to contain *Salmonella,* while analysis of celery by PCR showed the absence of *Salmonella* in 1,100 samples. Perhaps PCR testing of larger numbers of celery samples might have found some *Salmonella* positives, but celery testing was discontinued after 2004 to accommodate the testing of other, high-risk commodities, such as

TABLE 3. *Produce samples tested by MDP between 2002 and 2012, broken down by country of origin, percentage, and number of* Salmonella *isolates obtained*

Source of samples	No. (%) of samples	% *Salmonella* positive	No. of *Salmonella* isolates
United States (domestic)	82,582 (74)	0.13	123
Imported	25,667 (23)	0.07	22
Unknown	3,347 (3)	0.2	7
Imported from:			
Canada	1,796 (7)		0
Costa Rica	1,540 (6)		0
Guatemala	3,080 (12)		2
Honduras	1,540 (6)		0
Mexico	16,426 (64)		20
Others[a]	1,283 (5)		0

[a] Dominican Republic, Nicaragua, Peru, and others.

TABLE 4. *Percentages of commodity types tested by MDP between 2002 and 2012 that were imported and number of* Salmonella *isolates obtained from each commodity type*

Commodity	% imported	No. of *Salmonella* isolates
Cantaloupe	41	4
Celery	2	0
Cilantro	15	0
Green onions	65	5
Hot peppers	42	11
Lettuce	3	0
Parsley	12	1
Spinach	4	0
Sprouts	0	0
Tomatoes	44	1

sprouts, cilantro, and green onions *(51, 52)*. These limited sampling data, however, show that *Salmonella* can be present in celery, albeit at very low prevalence.

Salmonella was not detected by PCR in organically grown lettuce, but only 1,159 samples were tested, so the sample size was limited. It should also be cautioned that these data do not imply that the absence of *Salmonella* is in any way related to organic agricultural practices. Many studies in the United States have compared the microbiological quality of organically and conventionally grown preharvest fresh produce samples or spring salad mixes, and they did not find *Salmonella* in either type of samples *(32, 39)*. Similar studies from other countries also showed no *Salmonella* contamination in lettuce samples, either grown conventionally or organically *(31, 35)*.

The numbers of *Salmonella* PCR positives were slightly higher in bagged lettuce samples than in whole heads of lettuce (0.55% versus 0.36%). The same was noted between bagged and bunched spinach samples (0.58% versus 0.4%). It has been shown that postharvest handling of leafy produce, such as fresh-cut spinach, lettuce, and cilantro, exposes cut wound areas to which the pathogen can bind if present in contaminated wash water *(10, 21, 22)*. Therefore, it is possible that the slightly higher prevalence of *Salmonella* in bagged products may be due to adherence or other factors during the processing *(10, 22, 56)*.

***Salmonella* prevalence in imported produce.** In a 2008 outbreak of *Salmonella* Saintpaul in the United States, jalapeño peppers imported from Mexico were implicated *(6)*. A follow-up study on the microbial quality of serrano and jalapeño peppers in Mexico found that 1 of 40 jalapeño pepper samples tested was contaminated with *Salmonella* *(12)*. The MDP did not specifically target imported produce, nor did it use the country of origin as a sampling parameter, but the randomly collected samples included many imported commodities. By country of origin, 74% of the MDP samples were domestic products, including about 1% of the bagged lettuce samples that had mixed origins and were labelled as "United States and Canada" or "United States and Mexico" (Table 3). All alfalfa sprout samples were domestic, and so were most of the leafy greens, as imported lettuce and spinach accounted for only 3 to 4% of the samples of these produce types tested (Table 4). About 3% of the samples were of unknown origin, but 23% of the MDP samples were imported and the country of origin was identified (Table 3). Proportionally, 64% of the imported produce came from Mexico, followed by Guatemala, Canada, Honduras, Costa Rica, and others (Table 3). About 10 to 15% of the cilantro and parsley samples tested were imported, but over 40% of the cantaloupe and hot pepper and 65% of the green onion samples tested were imported (Table 4). Domestic produce comprised 74% of the samples tested by the MDP, and more *Salmonella* isolates were obtained from domestic produce (123 of 152, 81%) than from imported products (22 of 152, 14%), as expected. Similarly, a majority of imported produce samples were from Mexico, and they accounted for 20 of 22 *Salmonella* isolates obtained from imported products (Table 3), with 11 strains from hot peppers (5 serrano and 6 jalapeño), 1 each from green onions and cherry tomatoes, and the rest from other produce types.

The MDP data did not reveal any significant differences in the presence of *Salmonella* among imported samples, as low *Salmonella* prevalence was noted in both imported and domestic products. A Canadian study on produce imported from various sources, including from the United States, found no *Salmonella* in the imported samples *(3)*. Similarly, another study evaluated the microbial quality of domestic and Mexican produce, and no *Salmonella* was detected in any of the samples *(29)*. The presence of pathogens in fresh produce is highly unpredictable and varies depending on many factors aside from the place of origin. For example, 20% (31 of 152) of the MDP *Salmonella* isolates were from cilantro samples (Table 2), but while 15% of the samples were imported, all of the *Salmonella* isolates were obtained from domestic cilantro samples (Table 4).

***Salmonella* serotypes found in fresh produce.** The MDP survey found at least 51 different *Salmonella* serotypes associated with various produce commodities, but 6 of 152 strains (4%) were untypeable (Table 5). Characterization of ETEC and STEC isolates from fresh

TABLE 5. *Diversity of* Salmonella *serotypes found in produce tested by the MDP from 2002 to 2012*

Serotype, subspecies, or antigenic formula (total no. of isolates)	No. of isolates found in:									
	Cantaloupe	Celery	Cilantro	Green onions	Hot peppers	Lettuce	Parsley	Spinach	Sprouts	Tomatoes
Agona (1)				1						
Anatum (5)	2		1					1		1
Subsp. *arizonae* (IIIa) (12)			1		1			8	1	
Assen (1)			1							
Baildon (1)			1							
Bareilly (3)			2							1
C 2:d:−								1		
Carrau (2)	2									
Cerro (1)					1					
Cubana (14)									14	
Denver (2)					2					
Dessau (1)									1	
Subsp. *diarizonae* (IIIb) (2)			1			1				
Enteritidis (3)	1		1	1						
Florida (3)							3			
F (I) 11:nonmotile								1		
Gaminara (1)										1
Give (1)										1
Hartford (1)								1		
Havana (6)					2				4	
Infantis (8)			7			1				
Javiana (9)	2			4	3					
Kentucky (2)						1			1	
Lomalinda (1)			1							
Luciana (1)	1									
Mbandaka (2)								1	1	
Meleagridis (2)			1			1				
Michigan (1)					1					
Montevideo (4)			2						1	1
Muenchen (2)						1		1		
Newport (11)	2		1		3	1		4		
Norwich (1)			1							
Oranienburg (6)	2						1		2	
Ouakam (1)			1							
Paratyphi B (1)								1		
Poona (5)					1	2			2	
Redlands (1)			1							
Rottnest (1)			1							
Rubislaw (4)					2			1	1	
Saintpaul (4)			3		1					
Sandiego (1)	1									
Senftenberg (3)								1	2	
Thompson (2)						1			1	
Tucson (2)	1						1			
Typhimurium (3)	1	1				1				
Veneziana (1)									1	
I 6,8:d:−								1		
IV 43:z_4,z_{23}:− (2)						2				
IV 45:g,z_{51}:− (3)					1					
IV 48:g,z_{51}:−					2					
IV 50:z_4,z_2:− (1)					1					
Nontypeable			4			1				1
Total no. of isolates	15	1	31	6	21	13	5	22	32	6
Total no. of serotypes	10	1	18	3	13	11	3	13	13	6

TABLE 6. Salmonella *serotypes most commonly found by MDP in produce samples, the top five serotypes that cause foodborne outbreaks, and the common serotypes found in retail meat and in humans*

Isolates found in produce by MDP		Top 5 serotypes found in outbreaks	Serotypes found in:	
Serotype/subspecies	No. (%) isolated		Retail meat[a]	Humans
Cubana	14 (9.3)	Enteritidis	Typhimurium (CB)	Enteritidis
Subsp. *arizonae*/*diarizonae* (IIIa/IIIb)	12 (7.3)	Typhimurium	Newport (GB)	Typhimurium
Newport	11 (7.3)	Newport	Enteritidis (CB, GB)	Newport
Javiana	9 (6.0)	Javiana	Agona (GB)	Javiana
Infantis	8 (5.3)	Heidelberg	Anatum (GB)	I 4,[5],12:i:−
Havana	6 (4.0)		Dublin (GB)	Heidelberg
Oranienburg	6 (4.0)		Montevideo (CB, GB)	Montevideo
Anatum	5 (3.3)		Heidelberg (CB)	Saintpaul
Montevideo	4 (2.7)		Kentucky (CB)	Braenderup
Rubislaw	4 (2.7)		Seftenberg (CB)	Infantis
Saintpaul	4 (2.7)		Infantis (CB)	Paratyphi B

[a] CB, chicken breasts; GB, ground beef.

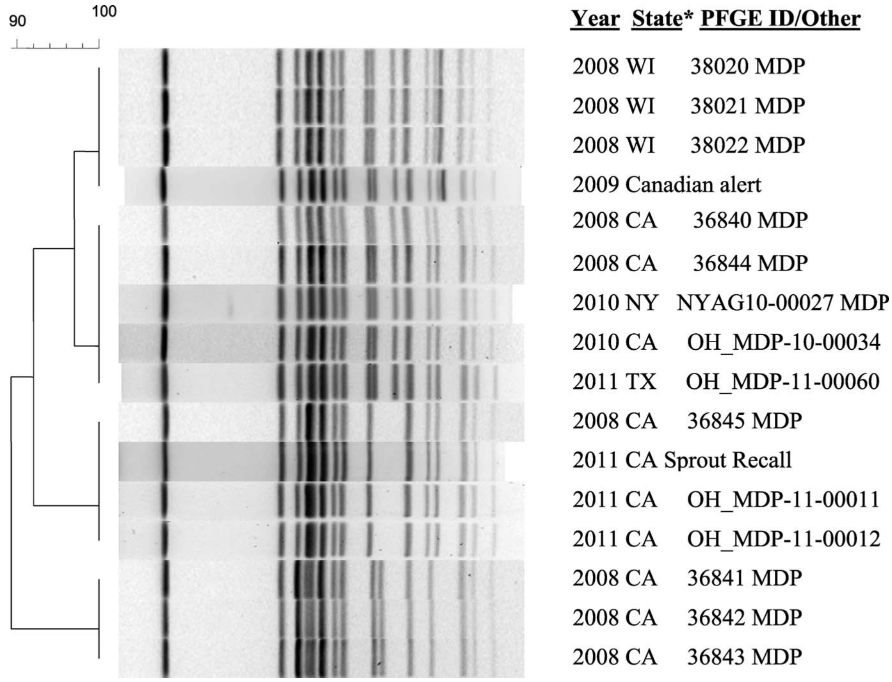
produce showed that 50 to 60% of the *E. coli* isolates were untypeable or had partial serotypes *(18, 19)*, so comparatively, the level of untypeable *Salmonella* strains in produce is low. Both cilantro and sprouts had over 30 *Salmonella* isolations, and each accounted for 20% of the total *Salmonella* isolates (Table 1), but the cilantro strains showed more diversity, with 18 serotypes, compared to 13 in sprouts (Table 5). Perhaps because cilantro is cultivated in soil, it is exposed to the environment and, therefore, can be expected to have a more diversified flora, whereas sprouts are grown hydroponically, so the sources of contamination are most likely from seeds and water. Aside from cultivation practices, variations in serotypes present in different commodities may also be due to differences in plant characteristics which may affect *Salmonella* adherence to the plant surfaces *(9, 24, 30)*. Also, it is not certain whether the culture medium used in selective enrichment might have created a bias toward enriching certain serotypes *(23, 58)*.

TABLE 7. *Antimicrobial resistance of* Salmonella *serotypes found in produce*

Commodity	No. of resistant isolates/total no. of isolates (%)	Serotype	Antibiotic resistance profile
Cantaloupe	1/15 (6.7)	Oranienburg	Chloramphenicol
Celery	0/1 (0)		
Cilantro	1/31 (3.2)	Montevideo	Nalidixic acid
Green onions	1/6 (16.7)	Agona	Tetracycline
Hot peppers	1/21 (4.8)	Havana	Trimethoprim, sulfamethoxazole
Lettuce	3/13 (23.1)	Thompson	Cephalothin
		Poona	Kanamycin
		Kentucky	Streptomycin, tetracycline
Parsley	1/5 (20)	Tucson	Amoxicillin, ampicillin, cefoxitin
Spinach	0/22 (0)		
Sprouts	1/33 (3)	Veneziana	Sulfisoxazole
Tomatoes	1/6 (16.7)	Unknown	Kanamycin

The most common serotypes found by MDP in produce were *S. enterica* serotype Cubana and *S. enterica* subspecies *arizonae* (IIIa), neither of which is common in retail meats or from human sources *(13, 33, 36, 43, 50)*, and they are not among the top 5 *Salmonella* serotypes that cause foodborne illness (Table 6) *(27, 41)*. All of the *Salmonella* Cubana isolates were from sprouts, and most of the *S. enterica* subsp. *arizonae* strains came from spinach. Among the other common produce serotypes found, *S. enterica* serotypes Newport, Javiana, and Infantis are also common in retail meat and human samples, and they are also implicated in salmonellosis infections that require hospitalization *(37)*. *Salmonella* Newport was isolated from cilantro, cantaloupe, lettuce, hot peppers, and spinach, while *Salmonella* Javiana was isolated from cantaloupe, green onions, and hot peppers. Clonal analysis of *Salmonella* Newport strains isolated from animals showed intraserotype genetic differences, and these strains grouped into distinct clades *(11, 27, 36)*. It would have been interesting to see whether the *Salmonella* Newport isolates from produce showed similar diversities. In the MDP study, *Salmonella* Infantis was mostly isolated from cilantro, and it is also common in retail meats and human samples, but it is not among the top serotypes that cause infections (Table 6). Other serotypes identified in produce, such as *S. enterica* serotypes Oranienburg, Anatum, Poona, Montevideo, and Rubislaw, were found in at least three produce types (Table 5), and some of these have had a history of causing outbreaks associated with produce. For example, *S. enterica* serotypes Javiana, Poona, Muenchen, Mbandaka, Senftenberg, and Litchfield accounted for more than 50% of the outbreaks associated with food plants *(27)*. Only *Salmonella* Litchfield was not isolated from produce by MDP. *Salmonella* Poona and Javiana are often associated with infections via reptile or amphibian contact *(27)*. Their presence in fresh produce suggests that these *Salmonella* serotypes, which have environmental, amphibian, or reptile reservoirs, may also be transmitted by fresh produce *(48)*. A single *S. enterica* serotype Paratyphi B was also isolated, from a spinach sample.

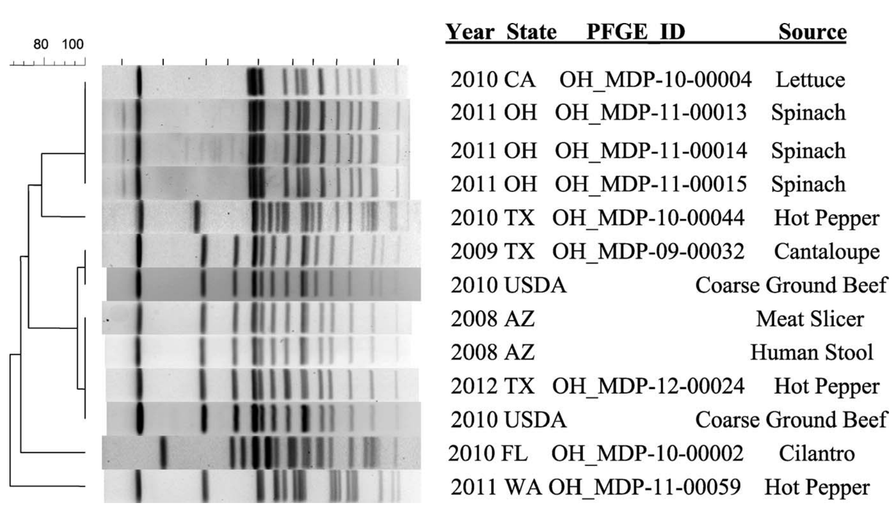
FIGURE 1. *PFGE profiles of MDP* Salmonella *Cubana isolates from alfalfa sprouts compared with those of human isolates from an outbreak and product recall. * State where sample was collected.*



The most common *Salmonella* serotypes that cause foodborne illness are *S. enterica* serotypes Typhimurium and Enteritidis, and both are commonly found in meat and eggs *(13, 16)*. These serotypes were found in a few samples of cantaloupes, cilantro, green onions, celery, and lettuce, but they were not very prevalent (Table 5). Still, all these produce types have been implicated in past outbreaks with both of these serotypes, so the MDP data are consistent with the idea that these serotypes can be found in fresh produce *(33, 52)*. Subtyping or genomic studies of these *Salmonella* serotypes that are common in produce may reveal their clonal origins and how they may be epidemiologically linked to serotypes from meat products or associated with outbreaks, as well as shed some insight on sources of contamination *(36, 50)*.

Antimicrobial resistance of *Salmonella* strains from produce. The CDC reported that 100,000 of the 1.2 million nontyphoidal *Salmonella* infections per year in the United States were caused by drug-resistant strains and raised the threat level to "serious" for resistance to cephalosporin and fluoroquinolone classes of antibiotics *(14)*. Consistent with those findings, about 30% of the *Salmonella* isolates obtained from human stool and blood samples between 1996 and 2007 showed resistance to one or more antibiotics, and the most common serotypes were *Salmonella* Enteritidis, Typhimurium, and Heidelberg *(16)*. Antibiotic resistance is also prevalent among *Salmonella* isolates from other sources. A study from New York State showed that 35.6% of the *Salmonella* isolates from humans and cattle exhibited antimicrobial resistance *(47)*. Another study showed that 84% of the *Salmonella* isolates obtained from retail meat in the Washington, DC, area were resistant to at least one antibiotic, and the most prevalent serotype was *S. enterica* serotype Agona *(59)*. Animal husbandry and meat production practices were thought to have contributed to the increased resistance in *Salmonella* strains from meat samples *(20, 59)*.

FIGURE 2. *PFGE profiles of MDP* Salmonella *Newport strains isolated from various types of fresh produce compared with those of* Salmonella *Newport isolates from human and meat samples.*



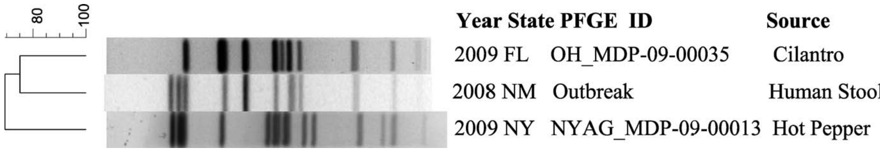


FIGURE 3. *PFGE profiles of MDP* Salmonella *Saintpaul isolates compared with that of a 2008 outbreak strain.*

Antibiotics are seldom used in crop production, but runoff from cattle farms may contaminate irrigation water, disseminating antibiotic-resistant bacteria onto the crops. For example, 16% of the STEC and ETEC strains isolated from produce by MDP between 2004 and 2012 had resistance to one or more antibiotics *(52)*. Little is known about the prevalence of antimicrobial-resistant *Salmonella* strains in produce. One study examined environmental samples in various California produce-growing regions and found that only 1 of 55 bacterial strains (1.8%) was resistant to antibiotics, and none of the strains was *Salmonella (24)*. The MDP study showed that 6.5% (10 of 152) of *Salmonella* isolates from produce showed resistance to antimicrobial compounds (Table 7). Except for lettuce samples, which harbored three different *Salmonella* serotypes resistant to different antibiotics, no particular produce type had a higher number of resistant strains. Among the three commodities from which most *Salmonella* were isolated, none of the spinach, either bunched or bagged samples, had any antibiotic-resistant *Salmonella,* and cilantro and sprouts both had one resistant strain each. Also, no *Salmonella* serotype was most commonly associated with antimicrobial resistance, and only one strain each of *S. enterica* serotypes Havana, Kentucky, and Tucson carried resistance to more than one antibiotic (Table 7). Hence, compared to *Salmonella* isolates from humans and other sources, the prevalence of antibiotic-resistant *Salmonella* in produce is fairly low.

PFGE profiles of *Salmonella* strains isolated from fresh produce. The PFGE profiles of all *Salmonella* strains isolated from produce by the MDP were uploaded to the CDC PulseNet database *(40)* and can be accessed using "MDP" as a prefix. All 14 *Salmonella* Cubana strains isolated by the MDP from alfalfa sprouts between 2008 and 2011 were compared with an isolate involved in a 2011 California sprout recall and a strain from a 2009 Canadian alert on contaminated sprouts (Fig. 1). The *Salmonella* Cubana strains shared about 90% similarity in their profiles (Fig. 1), but the profile of the MDP 2008 Wisconsin isolates was indistinguishable from that of the strain isolated from contaminated sprouts that resulted in the 2009 Canadian health hazard alert. Also, the profile of the MDP 2010 *Salmonella* Cubana strains isolated from New York and California was indistinguishable from that of a 2011 isolate from Texas, suggesting that these had a common seed source. Furthermore, the profile of a 2008 California isolate (CA 36845 MDP) was indistinguishable from that of the sprout isolate from the 2011 California recall, not only suggesting that the seed source was the same but also that the same *Salmonella* Cubana strain may have persisted in seeds for three years. *Salmonella* Newport has emerged as a common *Salmonella* serotype in meats *(11, 27, 43),* so the PFGE profile of the MDP produce isolates of *Salmonella* Newport were compared to those of isolates from ground beef or from cases of human illness. The profiles of a cluster of produce strains comprised of the MDP 2010 Texas isolates from cantaloupe and a 2012 Texas isolate from hot peppers were indistinguishable from those of a 2010 isolate from ground beef, a 2008 isolate from a meat slicer, and an isolate from a human stool sample (Fig. 2). Also, a 2010 MDP *Salmonella* Newport isolate from California lettuce was indistinguishable from a 2011 Ohio isolate from spinach, suggesting a common product source or, perhaps, that *Salmonella* Newport strains are genetically conserved. However, *Salmonella* Newport isolates from Texas hot peppers in 2010, Florida cilantro in 2010, and Washington hot peppers in 2011 all showed distinct PFGE profiles, indicative of genetic diversity among strains of this serotype. These results are consistent with the clade diversity that was reported *(11, 27, 36)* and show that *Salmonella* Newport strains are genetically diverse and that they may be cycled through beef and produce, perhaps due to the juxtaposition of the two agricultural operations and crop cultivation and animal husbandry practices *(27, 36, 44)*.

The PFGE profiles of some produce isolates could be matched to those of food, environmental, and human isolates, but it is more difficult to match the PFGE profiles of survey isolates to that of an outbreak strain. For example, in response to the 2008 *Salmonella* Saintpaul outbreak with hot peppers in New Mexico, the MDP screened 225 hot pepper samples, but no *Salmonella* was found *(52)*. In 2009, *Salmonella* Saintpaul was isolated from a Florida cilantro and a New York hot pepper sample, but these isolates only showed 70 to 75% similarity to the 2008 outbreak strain from New Mexico (Fig. 3) and so were not part of the outbreak. Marketed fresh produce tends to have broad areas of distribution, so it is not certain that targeting sample collection to the outbreak areas will find the outbreak strain. Even so, PFGE profiles of produce isolates can still provide useful epidemiological data. For example, the profiles of *Salmonella* Enteritidis isolates from a 2010 New York

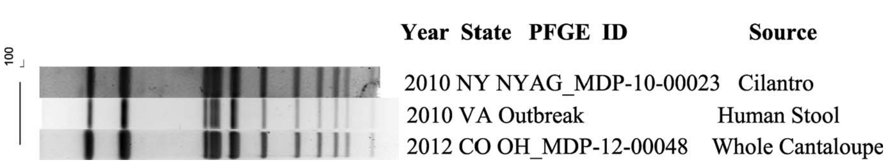


FIGURE 4. *PFGE profiles of MDP* Salmonella *Enteritidis isolates compared with that of a 2010 outbreak strain.*

cilantro sample and a 2012 Colorado cantaloupe sample were indistinguishable from that of a 2010 strain that caused an outbreak with shell eggs (Fig. 4), indicating that *Salmonella* strains can cycle through human infections and animal and produce sources.

In conclusion, the MDP data showed that the prevalence of *Salmonella* in fresh produce marketed in the United States is fairly low, and most isolates did not exhibit antimicrobial resistance. While this may be indicative that fresh produce in the United States is of good microbiological quality, it does not correlate with the increases in *Salmonella* illnesses and outbreaks associated with produce. However, even at low prevalence, the presence of *Salmonella* in produce can still have a broad impact on public health, as fresh produce is produced in large quantities, widely distributed, and almost always consumed raw. Some *Salmonella* serotypes found in produce are also common in other foods, and some are among the top five *Salmonella* serotypes that cause foodborne illness. In some cases, produce strains also had PFGE profiles that were identical to those of strains that caused outbreaks, suggesting that they can cycle through humans and animal and food sources, including produce. The MDP generated one of the largest publically available databases on the presence of *Salmonella* and other pathogens in a variety of fresh produce. Although the MDP has since been discontinued, it is critical that such monitoring efforts continue elsewhere to further our knowledge on the microbiological quality of fresh produce, which has had and will continue to have a great impact on consumer health and safety.

ACKNOWLEDGMENTS

The authors thank all the state agriculture laboratories that participated in the MDP. Their efforts and hard work generated this large and useful database, which has contributed greatly to our knowledge on the prevalence of pathogens in fresh produce.

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