

Distributions of *Salmonella* Subtypes Differ between Two U.S. Produce-Growing Regions

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***Salmonella* accounts for approximately 50% of produce-associated outbreaks in the United States, several of which have been traced back to contamination in the produce production environment. To quantify *Salmonella* diversity and aid in identification of *Salmonella* contamination sources, we characterized *Salmonella* isolates from two geographically diverse produce-growing regions in the United States. Initially, we characterized the *Salmonella* serotype and subtype diversity associated with 1,677 samples collected from 33 produce farms in New York State (NYS). Among these 1,677 samples, 74 were *Salmonella* positive, yielding 80 unique isolates (from 147 total isolates), which represented 14 serovars and 23 different pulsed-field gel electrophoresis (PFGE) types. To explore regional *Salmonella* diversity associated with production environments, we collected a smaller set of samples ($n = 65$) from South Florida (SFL) production environments and compared the *Salmonella* diversity associated with these samples with the diversity found among NYS production environments. Among these 65 samples, 23 were *Salmonella* positive, yielding 32 unique isolates (from 81 total isolates), which represented 11 serovars and 17 different PFGE types. The most common serovars isolated in NYS were *Salmonella enterica* serovars Newport, Cerro, and Thompson, while common serovars isolated in SFL were *Salmonella* serovars Saphra and Newport and *S. enterica* subsp. *diarizonae* serovar 50:r:z. High PFGE type diversity (Simpson's diversity index, 0.90 ± 0.02) was observed among *Salmonella* isolates across both regions; only three PFGE types were shared between the two regions. The probability of three or fewer shared PFGE types was <0.000001 ; therefore, *Salmonella* isolates were considerably different between the two sampled regions. These findings suggest the potential for PFGE-based source tracking of *Salmonella* in production environments.**

The genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori*, and represents approximately 2,600 known serovars. *S. enterica* has six subspecies, *enterica* (I) *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (V), and accounts for over 99% of *Salmonella* strains isolated worldwide (1). *S. enterica* subsp. *enterica* is the leading cause of bacterial food-borne illnesses, hospitalizations, and deaths in the United States (2). Approximately 95% of *S. enterica* infections in the United States are a result of consumption of contaminated foods (3, 4). *S. enterica* is estimated to be responsible for half of the produce-associated illnesses and a majority of produce-associated outbreaks in the United States (4–7). As a result, there is a need to identify likely sources of *Salmonella* contamination throughout the farm-to-fork continuum.

Subtyping is an important tool to detect food-borne outbreaks and to identify outbreak sources (8), as well as a powerful tool to investigate the diversity of food-borne pathogens in various hosts and environments. Even the most basic of subtyping methods (e.g., serotyping) can yield information on likely reservoirs for specific food-borne pathogens (9–11). For instance, *S. enterica* serovars Dublin and Choleraesuis are routinely associated with cattle and swine hosts, respectively (9, 12). In other studies (1, 13), *S. enterica* subsp. *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* were predominantly associated with cold-blooded animal hosts. *S. enterica* subsp. *diarizonae* was isolated most frequently from reptiles and amphibians obtained from the central coast of California (USA) (14). Another study (15) found that approximately 81% of pet snake fecal samples collected in Germany were positive for *S. enterica* subsp. *diarizonae* serovars. Other serovars are associated with a broad host range and a number of geographically diverse regions. *Salmonella* serovars Typhimurium and En-

teritidis are two of the most common serovars reported among human *Salmonella* isolates worldwide (16). A study conducted in Great Britain on wild-bird populations was able to identify two host-adapted *Salmonella* serovar Typhimurium strains by use of pulsed-field gel electrophoresis (PFGE) and phage typing (PT) (17), demonstrating that host-associated subtypes can be identified even in broad-host-range serovars.

While subtyping can yield information on likely potential hosts of *Salmonella*, studies (18–22) have also demonstrated the application of subtyping to track specific food-borne pathogen subtypes in the environment. One study (22) investigating the source of fecal pollution on a Japanese beach showed a strong association between *Enterococcus faecium* isolated in samples from the beach and one of the suspected contamination sources (a river that drains into the beach) by PFGE typing. Cooley et al. (18) used PFGE typing to track *Escherichia coli* O157:H7 in a California produce-growing region and found that the same PFGE type of *E. coli* O157:H7 was isolated from feral swine, cattle, surface water, sediment, and soil from one of the spinach farms that had been implicated in the 2006 spinach-borne *E. coli* O157:H7 outbreak. In

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TABLE 1 Summary of study data sets and *Salmonella* isolates^a

Data set no.	Region ^b	Isolation year(s)	Sample group or type	No. of samples	Frequency of positive samples (%)	No. of isolates subtyped ^c	No. of representative isolates ^d	No. of serovars	No. of PFGE types
I	NYS	2009–2011	Total group	588	27 (4.6) ^e	57	27	7	11
			Soil	178	4 (2.8)				
			Drag swab	175	3 (1.7)				
			Water	174	16 (9.2)				
			Fecal	61	4 (6.6)				
II	NYS	2012	Total group	600	26 (4.3)	35	29	9	11
			Soil	263	13 (4.9)				
			Drag swab	263	5 (1.9)				
			Water	74	8 (10.8)				
			Fecal	NC ^f					
III	SFL	2010	Total group	65	23 (35.4)	81	32	11	17
			Soil	8	5 (62.5)				
			Drag swab	8	3 (37.5)				
			Water	40	15 (37.5)				
			Fecal	9	0 (0)				
IV	NYS	2011	Total group	429	17 (4.0)	44	19	7	9
			Soil	90	2 (2.2)				
			Drag swab	219	4 (1.8)				
			Water	120	11 (9.2)				
			Fecal	NC					
V	NYS	2010	Total group	60	5 (8.3)	11	5	4	4
			Soil	20	2 (10)				
			Drag swab	20	0 (0)				
			Water	13	1 (7.7)				
			Fecal	7	2 (28.6)				
All			Total group	1,742	98 (5.8)	228	112	20	37
			Soil	559	26 (5.1)				
			Drag swab	685	15 (2.2)				
			Water	421	51 (12.1)				
			Fecal	77	6 (7.8)				

^a Data set I *Salmonella* isolates, serovars, and PFGE types have been previously described (26); data set II *Salmonella* isolates and serovars have been previously described (27); data set III is reported here; data set IV and V *Salmonella* isolates and serovars are unpublished. See File S1 in the supplemental material for further data set descriptions.

^b NYS, New York State; SFL, South Florida.

^c PFGE (using the restriction enzyme XbaI) was performed on one isolate per *Salmonella* positive sample for each isolation scheme (up to four isolates could be selected for the schemes RV-XLD, TT-XLD, RV-CHROMagar, and TT-CHROMagar).

^d Only isolates that were representative(s) of the *Salmonella* isolated in that sample were kept for further analyses (i.e., isolates from the same sample with identical serovars and PFGE types were excluded).

^e Isolates from one *Salmonella*-positive sample were unavailable for subtyping.

^f NC, not collected (a certain sample type was not collected for that data set).

another study, Patchanee et al. (20) reported that distinct *Salmonella* PFGE types were recovered from water samples collected from sites near swine production or forestry, residential/industrial sites, and agriculture cropland, further supporting that subtyping methods, such as PFGE typing, can be used to track *Salmonella* in the environment and to identify specific contamination sources.

Studies (20, 23–25) have characterized the distribution and diversity of *Salmonella* isolates from a number of different environments; however, there is minimal information on *Salmonella* in the produce production environment, and no one to our knowledge has compared *Salmonella* isolates obtained from two geographically diverse produce-growing regions using the same sample collection, detection, and isolation schemes. The purpose of this study was to characterize *Salmonella* isolates obtained from

environmental samples collected in produce production environments in New York State (NYS) and South Florida (SFL). We used both qualitative and quantitative methods to examine the distribution and diversity of *Salmonella* isolates from each region, as well as to compare the distribution of subtypes between these two regions. Additionally, *Salmonella* isolate subtype data were used to suggest potential sources of *Salmonella* contamination.

MATERIALS AND METHODS

Description of isolates used in this study. A total of 228 *Salmonella* isolates (147 isolates from NYS and 81 isolates from SFL) were assembled for this study using five *Salmonella* data sets, consisting of two published, two in preparation, and one reported here (Table 1; see File S1 in the supplemental material for further detailed descriptions). Four data sets (I, II, IV,

and V) representing NYS production environments had been collected to explore the association between *Salmonella* prevalence in produce fields and geographical and/or management factors (74 of 1,677 samples were *Salmonella* positive), whereas one data set (III) obtained from SFL was specifically collected for this study (23 of 65 samples were *Salmonella* positive) (Table 1; see also file S1). All sample collection and preparation (i.e., preparation of samples for *Salmonella* enrichment) were performed using the same methodology as previously described (26, 27). Detection and isolation of *Salmonella* were performed using a modified version of the Food and Drug Administration Bacteriological Analytic Manual (FDA BAM) method (28). Briefly, samples were diluted 1:10 with tryptic soy broth (TSB; Becton, Dickinson, Franklin Lakes, NJ) and incubated for 24 h at $35 \pm 2^\circ\text{C}$. Enrichment aliquots of 1.0 and 0.1 ml were transferred to tetrathionate (TT; Oxoid; Cambridge, United Kingdom) and Rappaport-Vassiliadis (RV; Oxoid) broths, respectively, and incubated for 24 h in a shaking water bath at $42 \pm 2^\circ\text{C}$. TT and RV cultures were plated onto xylose-lysine-deoxycholate agar (XLD; Neogen, Lansing, MI) and *Salmonella* chromogenic agar (CHROMagar; CHROMagar Company, Paris, France) and incubated for 24 and 48 h at 35 and $37 \pm 2^\circ\text{C}$, respectively. Presumptive *Salmonella* colonies (up to four colonies per isolation scheme, i.e., TT-XLD, RV-XLD, TT-CHROMagar, and RV-CHROMagar) were sub streaked to brain heart infusion (BHI; Becton, Dickinson) agar and incubated for 24 h at $37 \pm 2^\circ\text{C}$. One isolated colony was selected from each BHI agar plate and tested by a PCR assay for *invA*, which is specific to *Salmonella* (29). All PCR-confirmed *Salmonella* isolates were preserved at -80°C in 15% glycerol.

Isolates selected for molecular characterization. One isolate per isolation scheme (i.e., TT-XLD, RV-XLD, TT-CHROMagar, and RV-CHROMagar) was selected for serotyping and PFGE typing (147 isolates from NYS and 81 isolates from SFL, for a total of 228 isolates). This approach was used to capture all potential strains of *Salmonella* that may be present in a sample as several studies (14, 17, 23, 27, 30, 31) have shown that multiple *Salmonella* strains may be isolated from the same sample.

Traditional serotyping. Serotyping was performed on all 228 isolates by the Wadsworth Center, New York State Department of Health (Albany, NY), using the White-Kauffman-Le Minor scheme (1).

Pulsed-field gel electrophoresis. PFGE typing was performed on all 228 isolates using the standard U.S. Centers for Disease Control and Prevention (CDC) PulseNet protocol (32). Briefly, *Salmonella* cells were embedded in 1% SeaKem Gold agarose (Lonza, Rockland, ME), lysed, washed, and digested with 50 U/plug of XbaI (Roche Applied Science, Indianapolis, IN) at 37°C . Separation of the restricted DNA fragments was performed by a Chef Mapper XA (Bio-Rad, Hercules, California) for 18 to 20 h in 1% agarose gels. Voltage was set to 6 V/cm with an initial switch time of 2.16 s and a final switch time of 63.8 s. *Salmonella* serovar Braenderup (strain H9812) was used as the reference standard to allow normalization and comparison of gel images (33). Gel images were captured by a Bio-Rad Gel Doc using Multi-Analyst software, version 1.1 (Bio-Rad). PFGE images were analyzed by BioNumerics software, version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium). Similarity clustering analyses were performed using the unweighted pair group method with arithmetic mean algorithm (UPGMA) based on Dice coefficients with a maximum space tolerance of 1.5%. PFGE types were named using Cornell University Food Safety Laboratory (CUFSL) nomenclature (34): laboratory location of New York Cornell University (NYCU), genus *Salmonella* (JAA), enzyme XbaI (X01), and pattern code (four-digit number). Four-digit pattern codes were assigned by comparison of patterns to an internal reference database (CUFSL, Ithaca, NY) comprised of approximately 6,000 *Salmonella* isolates. Lastly, PFGE was used to predict the serovar of a given isolate by comparison to the CUFSL database using a similarity cluster analysis (as described above) (35).

Molecular serotyping. Molecular serotyping was performed on 12 isolates where serovars reported by traditional serotyping did not match serovars predicted by PFGE. It has been reported in one study (36) that approximately 10% of *S. enterica* subsp. *enterica* isolates ($n = 754$ tested)

were incorrectly serotyped by traditional serotyping, and thus molecular serotyping may aid in identification (37). Serovar was confirmed by PCR detection of the *Salmonella* O serogroup genes by a multiplex PCR assay that simultaneously targets genes for five *Salmonella* O antigens (B, C1, C2–C3, D1, and E1), as described by Ranieri et al. (35) and Herrera-Léon et al. (38); in addition, PCR amplification and sequencing of the genes encoding the H1 and H2 antigens were performed. Primer sets, DNA amplification, and PCR conditions for *fliC* (encodes H1 antigen) and *fliB* (encodes H2 antigen) have been previously described by Imre et al. (39) and Mortimer et al. (40). DNA sequence data were compared to sequences in an internal database (CUFSL) of H1 and H2 sequences, as previously described (35). Molecular serotyping results were able to resolve serovar conflicts for the 12 isolates with different serovars identified by traditional serotyping and PFGE. Discrepancies between traditional serotyping and molecular-based methods can be due to a variety of reasons, including auto-agglutination or cross-reactivity of antisera, or can occur in mucoid strains (where the O antigen may be masked) (35, 41, 42). In all cases, the serovar predicted by PFGE matched the serovar identified by molecular serotyping results.

Final *Salmonella* isolate data set. Upon completion of serotyping and PFGE typing, only isolates that were “unique” (i.e., defined as isolates from a given sample with different serotype-PFGE pattern combinations) were kept for further analyses; isolates from the same sample with identical serovars and PFGE types were excluded from further analysis (Table 1 gives isolate details, such as the number of isolates subtyped and the number of representative isolates yielded). This approach yielded 112 unique *Salmonella* isolates (80 from NYS and 32 from SFL).

Statistical analysis. All analyses were performed in the statistical computing environment R, version 3.0.2 (43). Diversity of PFGE types between the NYS and SFL regions was assessed using two assessment tools: a diversity index and probability simulations.

Simpson’s index of diversity (D) was calculated for PFGE types found among all unique isolates and among unique isolates for each region (44), with 95% confidence intervals (45). D values closest to 1 indicate a high diversity, while D values closest to 0 indicate a low diversity. To test if isolates from NYS and SFL were drawn from two distinct populations, we performed simulations to quantify the likelihood of three or fewer shared subtypes occurring between the regions, given that all the *Salmonella* isolates were drawn from one population. Briefly, we randomly permuted the isolates across the two regions and computed the number of shared PFGE types. The simulation was performed 1,000,000 times, and the probability of simulations (i.e., permutations) with three or fewer shared subtypes was calculated.

Data access. Isolate information and subtyping data from this study are archived and available through the Cornell University Food Microbe Tracker database (<http://www.foodmicrobetracker.com>).

RESULTS AND DISCUSSION

This study is one of the first to characterize *Salmonella* distribution and diversity associated with the produce production environment in NYS. In addition, we assembled a smaller set of *Salmonella* isolates from SFL that were characterized to examine the regional distribution of subtypes between NYS and SFL. In the NYS data set, 74 of 1,677 previously collected environmental samples were *Salmonella* positive (Table 1). In the smaller SFL data set, 23 of 65 previously collected environmental samples were *Salmonella* positive (Table 1). The difference in *Salmonella* prevalence rates between the NYS and SFL data sets (approximately 4.5 and 35%, respectively) may be due to several factors, such as differences in environmental pressures, agricultural practices, wildlife, and/or weather. While neither our study nor previous studies (14, 24, 46, 47) were designed to identify the exact reasons for these differences in *Salmonella* prevalences on a regional scale, these findings suggest the need for future studies to investigate what

TABLE 2 Serovars and PFGE types found among *Salmonella* isolates from study samples collected in New York State and South Florida U.S. produce production environments

<i>Salmonella</i> organism ^a	PFGE type ^b	No. of isolates by region and data set:					
		NYS					SFL III
		I	II	IV	V	Total	
<i>S. enterica</i> subsp. <i>enterica</i> serovars							
Agona	NYCU,JAAX01.1131		3			3	
Baildon	NYCU,JAAX01.0345						2
Braenderup	NYCU,JAAX01.1196						1
Cerro	NYCU,JAAX01.0213	10	5		1	16	
Enteritidis	NYCU,JAAX01.1225			4		4	
Gaminara	NYCU,JAAX01.1202						1
Give	NYCU,JAAX01.1215	1				1	
	NYCU,JAAX01.1216	1	2			3	
	NYCU,JAAX01.1217			1		1	
	NYCU,JAAX01.1218			1		1	
Infantis	NYCU,JAAX01.1203			2		2	
Litchfield	NYCU,JAAX01.1197						1
	NYCU,JAAX01.1198						1
Newport	NYCU,JAAX01.0121		6			6	
	NYCU,JAAX01.0126		1			1	
	NYCU,JAAX01.0296	2	2	2	1	7	
	NYCU,JAAX01.1212	1		4		5	
	NYCU,JAAX01.1213						1
	NYCU,JAAX01.1221	3				3	2
	NYCU,JAAX01.1222						3
	NYCU,JAAX01.1223						1
Rubislaw	NYCU,JAAX01.1201						3
	NYCU,JAAX01.1220			1		1	
Saphra	NYCU,JAAX01.1194						7
Senftenberg	NYCU,JAAX01.1005		1			1	
Tennessee	NYCU,JAAX01.1214		1			1	
Thompson	NYCU,JAAX01.0157	4	5	1	2	12	1
	NYCU,JAAX01.1199	1				1	1
	NYCU,JAAX01.1200						1
Typhimurium	NYCU,JAAX01.0072						1
	NYCU,JAAX01.1207		1			1	
	NYCU,JAAX01.1208	1				1	
4,5,12:i:–	NYCU,JAAX01.1209						2
6,8:i:–	NYCU,JAAX01.0096	1				1	
<i>S. enterica</i> subsp. <i>diarizonae</i> serovar							
50:r:z	NYCU,JAAX01.1210			3		3	
	NYCU,JAAX01.1211						3
<i>S. enterica</i> subsp. <i>houtenae</i> serovar							
40:z4,z32:–	NYCU,JAAX01.1219	2	2		1	5	

^a Serotyping was performed by agglutination at the Wadsworth Center, New York State Department of Health.

^b PFGE was performed in accordance with the standard CDC PulseNet protocol for *Salmonella* using the restriction enzyme XbaI (32). PFGE types were named with Cornell University Food Safety Laboratory (CUFSL) nomenclature and were assigned by comparison of PFGE patterns to an internal reference database (CUFSL, Ithaca, NY) comprised of approximately 6,000 *Salmonella* isolates. PFGE types in boldface were found in both NYS and FL.

factors may influence differences in pathogen prevalences between regions. Upon completion of serotyping and PFGE typing, the combined NYS and SFL data sets consisted of 112 unique *Salmonella* isolates (Table 2), specifically, 80 isolates from NYS and 32 isolates from SFL. Overall, multiple *Salmonella* serovars were isolated from a number of individual NYS and SFL samples, further supporting the importance of using multiple enrichment

TABLE 3 Two or more serovars that were isolated from *Salmonella*-positive samples by the four different detection and isolation schemes

Sample region and no. ^a	Sample type	<i>S. enterica</i> serovar or subsp. by isolation scheme ^b			
		RV		TT	
		CHROMagar	XLD	CHROMagar	XLD
NYS					
1	Soil	Newport			Thompson
2	Soil	Senftenberg	Newport		
3	Drag swab	Tennessee	Agona		
4	Water				Typhimurium
5	Water			Enteritidis	Give
6	Water	Newport			<i>diarizonae</i>
SFL					
7	Soil	Braenderup			Litchfield
8	Soil		Newport		Saphra
9	Drag swab				Thompson
10	Drag swab	Baildon			Thompson
11	Water	Newport			Saphra
12	Water	<i>diarizonae</i>	Thompson		
13	Water	Rubislaw			Gaminara
14	Water	Saphra	<i>diarizonae</i>		Newport

^a NYS, New York State; SFL, South Florida.

^b Enrichment media are Rappaport-Vassiliadis (RV) and tetrathionate (TT) broths; plating media are *Salmonella* chromogenic agar (CHROMagar) and xylose-lysine-deoxycholate agar (XLD).

and plating schemes for detection and isolation of *Salmonella*. Additionally, across both NYS and SFL data sets, we observed a number of different serovars that previously have been associated with certain hosts, suggesting in some cases that *Salmonella* contamination in production environments could be linked to a probable source through use of subtyping. Lastly, we showed a distinct difference between *Salmonella* subtypes isolated from the sampling areas in two produce-growing regions (NYS and SFL), suggesting that regional characteristics, such as specific landscapes, climates, and/or wildlife populations influence the *Salmonella* subtype diversity found in different produce production environments.

Multiple serovars were isolated from *Salmonella*-positive samples. Serotyping and PFGE typing were performed on one isolate per isolation scheme from the 97 *Salmonella*-positive samples (74 and 23 *Salmonella*-positive samples from NYS and SFL, respectively); while our data set represented 98 *Salmonella*-positive samples (Table 1), isolates from one *Salmonella*-positive sample from NYS data set I were unavailable for subtyping (preservation failed). Among the 97 *Salmonella*-positive samples evaluated, 83 samples yielded one serovar (92 and 65% of *Salmonella*-positive samples from NYS and SFL, respectively, yielded only one serovar), and 14 samples yielded two or more serovars. The 14 *Salmonella*-positive samples that yielded two or more serovars represented 6 samples from NYS and 8 samples from SFL (Table 3); for each of the 6 samples from NYS, two different serovars were isolated. Among the SFL samples, two serovars were isolated from seven samples, and three serovars (*Salmonella* serovar Newport, *Salmonella* serovar Saphra, and *S. enterica* subsp. *diarizonae* serovar 50:r:z) were isolated from one sample (Table 3). While our study found multiple serovars in some samples by subtyping only one isolate per isolation scheme, it may be warranted in future studies to test multiple colonies per isolation scheme to further investigate potential polyclonal contamination.

Several studies (14, 17, 23, 47) have isolated multiple *Salmo-*

nella serovars from one sample. For instance, Jokinen et al. (23) isolated more than one serovar from all *Salmonella*-positive water samples ($n = 29$) collected from a Canadian watershed. In our study, 50% of the *Salmonella*-positive samples with multiple serovars represented water samples (3/6 and 4/8 positive samples from NYS and SFL, respectively). Our water sample volume tested was 250 ml, while in the Jokinen et al. study the water sample volume tested was 500 ml. The number of serovars isolated from each sample may be influenced by the sample volume tested; however, further studies are required to correlate the volume of water tested and the likely number of serovars. Isolation of multiple serovars from water samples may also be more likely because *Salmonella* is more uniformly dispersed in water than in other sample types in our study (e.g., soil samples; 25 g).

Multiple *Salmonella* enrichment and plating schemes were used here to facilitate detection of *Salmonella* with atypical phenotypic characteristics as previous studies (30, 31, 48–51) have shown that detection of certain serovars of *Salmonella* may be influenced by enrichment and plating media. In our study, the 14 *Salmonella*-positive samples where multiple serovars of *Salmonella* were isolated yielded 14 different serovars (Table 3). Overall, no apparent association was observed between isolation of a *Salmonella* serovar and a specific enrichment and plating scheme (Table 3). For example, *S. enterica* subsp. *diarizonae* serovar 50:r:z was isolated from three of the four enrichment and plating schemes (RV-CHROMagar, RV-XLD, and TT-XLD). Similarly, *Salmonella* serovar Saphra was also isolated from three of the four schemes (TT-CHROMagar, RV-CHROMagar, and TT-XLD). Other serovars that were isolated more than once were also isolated from two or more schemes. Select strains of four of the serovars isolated here (*Salmonella* serovars Thompson, Enteritidis, and Typhimurium and *S. enterica* subsp. *diarizonae*) have been reported to be weak H₂S producers. No evident association was observed between serovars that have been reported as weak H₂S producers and a specific scheme (Table 3). In this study, we employed one plating medium that does (XLD) and one that does not (CHROMagar) use the production of H₂S as an indicator of *Salmonella* (49, 52). Select strains of six of the serovars isolated here (*Salmonella* serovars Tennessee, Newport, Agona, Senftenberg, and Typhimurium and *S. enterica* subsp. *diarizonae*) have been reported to ferment lactose (53–55). No apparent association was observed here between the serovars that have been reported to ferment lactose and a specific enrichment and plating scheme (Table 3). Even though our sample size was small (14 cases of isolation of multiple *Salmonella* serovars from one sample), our results further support the idea that the use of different enrichment and plating schemes is needed to detect and isolate different *Salmonella* subtypes that may be present in a sample.

***Salmonella* serovars isolated from produce-growing regions were diverse.** We identified 20 different serovars among the 112 subtyped unique isolates from NYS and SFL (Table 2). Specifically, 14 and 11 serovars were identified among the 80 NYS and 32 SFL isolates, respectively; 5 serovars were found among isolates from both regions. *S. enterica* subspecies identified across both NYS and SFL data sets were *enterica* (18 serovars), *diarizonae* (1 serovar), and *houtenae* (1 serovar) (Table 2). While *S. enterica* subsp. *enterica* serovars are often associated with warm-blooded animal hosts and are the most commonly associated with human *Salmonella* infections linked to foods (56–58), a few documented cases have linked cold-blooded animal hosts (e.g., reptiles and

amphibians) to *S. enterica* subsp. *enterica* infections (9, 59, 60). Serovars of *S. enterica* subsp. *diarizonae* and *S. enterica* subsp. *houtenae* are primarily associated with cold-blooded animal hosts (1, 13, 14) and are most commonly associated with human *Salmonella* infections linked to reptiles (15, 61). In the NYS data set, isolates identified as *S. enterica* subsp. *enterica* represented *Salmonella* serovars Newport (22 isolates), Cerro (16 isolates), Thompson (13 isolates), Give (6 isolates), Enteritidis (4 isolates), Agona (3 isolates), Typhimurium (2 isolates), and Infantis (2 isolates) as well as 6,8:i:–, Rubislaw, Senftenberg, and Tennessee (1 isolate each); in addition *S. enterica* subsp. *diarizonae* serovar 50:r:z (3 isolates) and *S. enterica* subsp. *houtenae* serovar 40:z4,z32:– (5 isolates) were identified (Table 2). In the SFL data set, isolates identified as *S. enterica* subsp. *enterica* represented *Salmonella* serovars Newport (7 isolates), Saphra (7 isolates), Rubislaw (3 isolates), Thompson (3 isolates), 4,5,12:i:– (2 isolates), Litchfield (2 isolates), and Baildon (2 isolates), as well as *Salmonella* serovars Braenderup, Gaminara, and Typhimurium (1 isolate each); in addition *S. enterica* subsp. *diarizonae* serovar 50:r:z (3 isolates) was identified (Table 2). *Salmonella* serovars Newport, Cerro and Thompson were isolated most frequently in NYS and represented approximately 28, 20, and 16% of the NYS isolates (Table 2). *Salmonella* serovars Newport and Saphra were isolated most frequently in SFL, and each represented 22% of the SFL isolates (Table 2). All other serovars represented <10% of the NYS and SFL isolates.

Among the *Salmonella*-positive samples obtained from NYS and SFL, *Salmonella* serovar Newport was isolated the most (Fig. 1, green line) (22/80 isolates NYS and 7/32 isolates SFL). *Salmonella* serovar Newport has been isolated from a broad range of hosts (e.g., dairy cattle, snakes, and hedgehogs) and from a number of diverse environmental sources (e.g., soil and water) (20, 62). Additionally, *Salmonella* serovar Newport is one of the most frequently reported serovars among human *Salmonella* isolates in North America, Europe, and Latin America (16, 63) and is associated with a wide range of foods and animals (e.g., beef and poultry) (58, 64). Produce-borne outbreaks of *Salmonella* serovar Newport have been routinely traced back to the produce production environment (65, 66) and have been associated with numerous commodities, including lettuce, mangoes, melons, alfalfa sprouts, and tomatoes (4, 67). For example, in 2005, tomatoes from Virginia were implicated as the food vehicle for an outbreak of *Salmonella* serovar Newport, with an estimated 72 illness cases across 16 states. The source of this outbreak was traced back to a pond that served as an irrigation source for the tomato fields (66). The identification of *Salmonella* serovar Newport in both regions highlights the widespread nature of this serovar; furthermore, the number of *Salmonella* serovar Newport outbreaks associated with different produce commodities emphasizes the importance of this serovar in produce production environments.

Salmonella serovar Cerro was isolated exclusively from NYS (Fig. 1, orange line) (16/80 isolates). Previous studies (68–70) have indicated that *Salmonella* serovar Cerro is prevalent in Pennsylvania (USA) and NYS, especially in dairy production environments. This suggests that *Salmonella* serovar Cerro may be a likely contaminant in produce production environments near dairy cattle operations. Yet *Salmonella* serovar Cerro has rarely been associated with human *Salmonella* infections and has been isolated from asymptomatic, healthy children in India (71). Of the few documented *Salmonella* serovar Cerro outbreaks, the most nota-

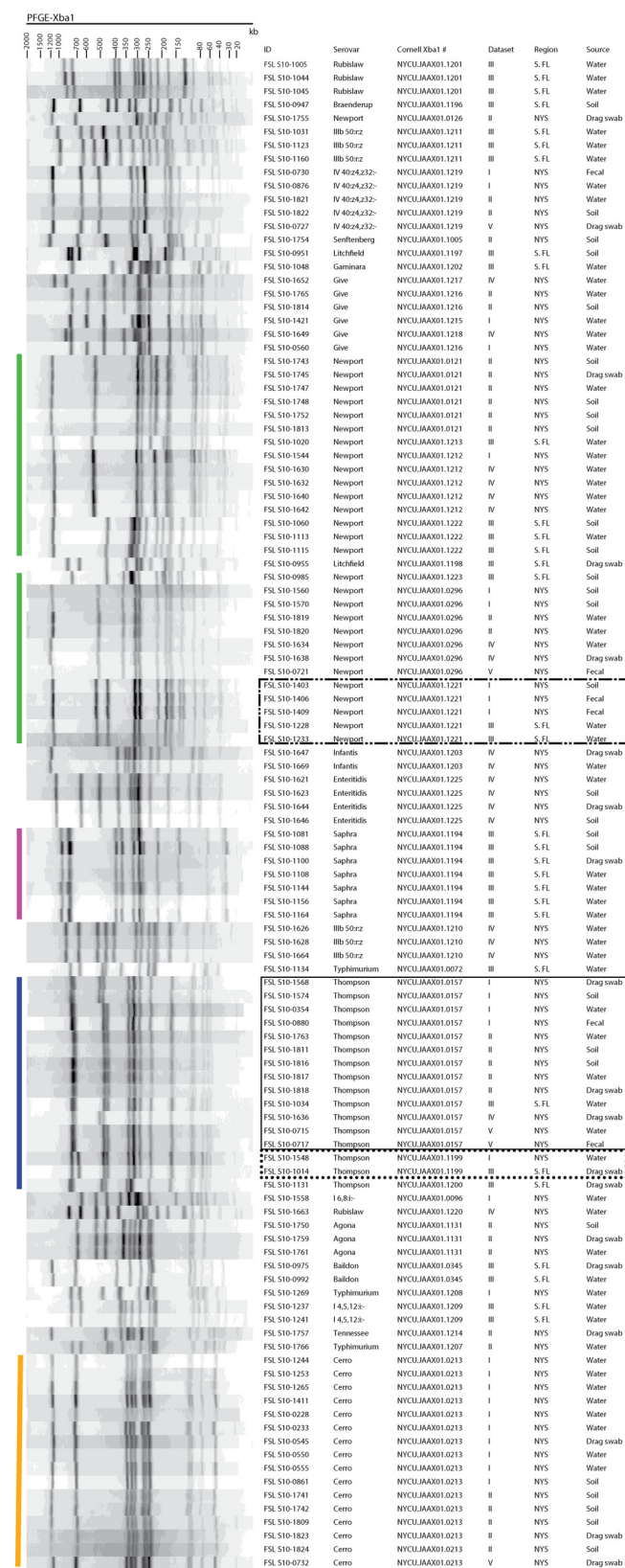


FIG 1 XbaI PFGE patterns for the representative 112 *Salmonella* isolates from environmental samples obtained from New York State and South Florida produce production environments. Band sizes (kb) are displayed at the top of the PFGE pattern images. The PFGE pattern order displayed is the result of

ble occurred in New Mexico (USA) (29 illnesses and 7 hospitalizations) and was linked to consumption of contaminated carne seca (71).

Salmonella serovar Thompson was isolated from both NYS and SFL (Fig. 1, blue line) (13/80 isolates from NYS and 3/32 isolates from SFL). One field study (72) conducted in NYS found 10% (13/129) of surface water samples tested positive for *Salmonella* serovar Thompson. Of the 13 isolates identified as *Salmonella* serovar Thompson from NYS in our study here, 5 were isolated from surface water samples. This serovar has been associated with two produce-borne outbreaks, a 1999 outbreak with 41 reported cases linked to the consumption of contaminated cilantro in California (USA) (73) and a 2004 outbreak with 21 cases linked to rucoleta lettuce in Norway (74). *Salmonella* serovar Thompson is common among human salmonellosis cases, as evident by its rank of 13 in the CDC's top 20 *Salmonella* isolates from human sources (56).

Salmonella serovar Saphra was isolated exclusively from SFL and represented 22% of the SFL *Salmonella* isolates (Fig. 1, purple line) (7/32 isolates). This serovar is not commonly isolated from humans or animals, and minimal information has been reported on its ecology in the environment. Two studies, conducted in Argentina (75) and Brazil (76), isolated *Salmonella* serovar Saphra from surface water and animal drinking water samples. There has been only one documented outbreak of *Salmonella* serovar Saphra in the United States, with 24 illnesses and 5 hospitalizations, due to consumption of contaminated cantaloupe (77), purchased by a single distributor who obtained the cantaloupe from a specific region in Mexico.

We identified a number of *Salmonella* serovars that have previously been linked to produce-borne outbreaks; however, we also identified serovars that are rarely linked to human cases of salmonellosis (e.g., *Salmonella* serovar Cerro). This finding suggests a need for future studies to investigate the differences in virulence between different *Salmonella* serovars (and in some cases virulence between different strains of the same serovar). In addition, we observed several serovars that are associated with specific hosts, such as *S. enterica* subsp. *diarizonae* serovar 50:r:z, which is commonly associated with reptile and amphibian hosts. Additional studies are needed to further our understanding of various *Salmonella* serovars and their association with different hosts in produce production environments, especially geographical locations in the United States and abroad.

***Salmonella* PFGE types show significant differences between regions.** To explore the regional *Salmonella* diversity associated with the produce production environment, we also performed PFGE on the *Salmonella* isolates from NYS ($n = 80$) and SFL ($n =$

BioNumerics similarity analyses using the unweighted pair group method with arithmetic mean algorithm (UPGMA) and the Dice correlation coefficient with a maximum space tolerance of 1.5%. ID is the isolate designation, serovar # is the PFGE type assigned by comparison of PFGE patterns to the Cornell University Food Safety Laboratory (CUFSL; Ithaca, NY) database of 6,000 *Salmonella* isolates, data set indicates the study origin for the isolate, region is New York State (NYS) or South Florida (SFL), and source is the type of sample for the isolate. *Salmonella* serovars discussed in the text are labeled by color: green, Newport; purple, Saphra; blue, Thompson; orange, Cerro. Boxes represent isolates from NYS and SFL that share PFGE types: dashed box, NYCU.JAAX01.1221 (*Salmonella* serovar Newport); solid box, NYCU.JAAX01.0157 (*Salmonella* serovar Thompson); dotted box, NYCU.JAAX01.1199 (*Salmonella* serovar Thompson).

32). We identified 37 unique XbaI-PFGE types among these 112 *Salmonella* isolates (Table 2). Isolates from NYS and SFL represented 23 and 17 different PFGE types, respectively; only 3 PFGE types were found among isolates from both regions (Table 2 and Fig. 1). A high level of PFGE type diversity was observed among all isolates ($D = 0.90 \pm 0.02$) as well as within each region ($D = 0.92 \pm 0.03$ and $D = 0.93 \pm 0.05$ for NYS and SFL, respectively). The largest number of PFGE types was observed among *Salmonella* serovar Newport isolates (eight unique PFGE types among 29 *Salmonella* serovar Newport isolates). Of the eight *Salmonella* serovar Newport PFGE types, four and three types each were observed exclusively in the NYS and SFL regions, respectively, and one PFGE type was shared between the two regions (Table 2 and Fig. 1, dashed box). Previously, a specific *Salmonella* serovar Newport subtype had been repeatedly isolated over a 10-year span from the eastern shore of Virginia (USA) (66). This *Salmonella* serovar Newport subtype has been associated with at least two known outbreaks, linked to tomatoes harvested in Virginia (66), in addition to being isolated from several waterfowl and non-waterfowl fecal samples collected from the eastern shore of Virginia (78). These findings suggest that specific *Salmonella* serovar Newport subtypes may be associated with certain regions. Additionally, in our study, different PFGE types of *S. enterica* subsp. *diarizonae* serovar 50:r:z were identified from the NYS and SFL regions sampled (Fig. 1). These data may indicate carriage of different *Salmonella* strains by reptile or amphibian populations in these regions; further studies could explore whether different reptile or amphibians in these regions carry distinct *Salmonella* subtypes. One study conducted in Mississippi (USA) observed a strong association between patients infected with *Salmonella* serovar Javiana and contact with amphibian species endemic to the southeastern United States (79). Gorski et al. (14) also found several *Salmonella* strains, all with the same PFGE type, repeatedly isolated from cold-blooded vertebrates and surface water in the same region in California. Overall, a number of studies, including our data reported here, suggest that some *Salmonella* subtypes may be more prevalent in certain areas, possibly due to persistence in animal and human host populations or in abiotic environments or due to their adaptation to specific hosts or environments found in a given region.

Only three PFGE types were shared between the two regions, *Salmonella* serovar Thompson patterns NYCU.JAAX01.0157 (Fig. 1, solid box) and NYCU.JAAX01.1199 (Fig. 1, dotted box) and *Salmonella* serovar Newport pattern NYCU.JAAX01.1221 (Fig. 1, dashed box). A-1,000,000 iteration simulation calculated the probability of three or fewer shared PFGE types being observed by chance between the two regions as <0.000001 , indicating a distinct difference in *Salmonella* PFGE types between isolates recovered in the NYS and SFL regions sampled. *Salmonella* isolates from NYS were obtained over a 3-year period (2010 to 2012) and represented a number of produce production environments across the state and fields planted with a number of crop types (e.g., cabbage, peppers, summer squash, and melons) (see File S1 the supplemental material). *Salmonella* isolates from SFL were obtained at one time point (December 2010) and represented a small subset of produce production environments and a single crop type (leafy greens) (see File S1). As our study did not specifically examine the association between specific produce production environments, including crop types, farm-specific management practices, or sampling time and *Salmonella* subtype diversity, future studies

are needed to determine how these and other factors may influence regional *Salmonella* ecology and serotype diversity. Therefore, one cannot easily extrapolate from our findings that show subtype differences between the two sampled regions, NYS and SFL, with distinct overall environmental conditions to other sampling areas or regions. Other studies have also reported meteorological events (23, 26, 46) and/or field management practices (27, 80, 81) to influence pathogen prevalence in produce production environments; whereas the effects of these factors on subtype diversity still remain to be explored, it is feasible that subtypes isolated may differ by season (e.g., as season may have effects on movement or presence of wildlife hosts). While many factors can clearly affect *Salmonella* subtype diversity, it is still important to note that a number of previous studies (23–25, 46, 47) in regions within the United States and Canada have reported *Salmonella* serovar diversity distinct from that reported here, supporting the idea that *Salmonella* subtype diversity may differ by region and sampled area. For example, one study (25) found that *Salmonella* isolated from water samples in the Upper Suwannee River Basin in North Florida (30 isolates, representing 8 serovars) represented several different serovars from those reported here for isolates from SFL. Other studies found that *S. enterica* subsp. *arizonae* represented 40% of the *Salmonella* isolates from a watershed in Georgia (USA) (47) and that *Salmonella* serovar Rubislaw was isolated most frequently from water samples obtained from a Canadian watershed (23). Furthermore, a study investigating the distribution and diversity of *Salmonella* in a produce-growing region in California primarily isolated *Salmonella* serovar Give (46). Our findings, in concert with previously discussed studies, demonstrate the potential for PFGE-based source tracking of *Salmonella* as *Salmonella* subtypes can be highly associated with certain hosts (e.g., *Salmonella* serovar Cerro and cattle) and environments (e.g., *Salmonella* serovar Newport and the eastern shore of Virginia). These data also suggest that food safety in the produce production environment may require efforts to tailor preventive controls that account for unique food safety hazards and contamination routes associated with different areas and regions.

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