

# Diversity of *Salmonella* Isolates from Central Florida Surface Waters

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Identification of *Salmonella* serotypes is important for understanding the environmental diversity of the genus *Salmonella*. This study evaluates the diversity of *Salmonella* isolates recovered from 165 of 202 Central Florida surface water samples and investigates whether the serotype of the environmental *Salmonella* isolates can be predicted by a previously published multiplex PCR assay (S. Kim, J. G. Frye, J. Hu, P. J. Fedorka-Cray, R. Gautom, and D. S. Boyle, *J. Clin. Microbiol.* 44:3608–3615, 2006, <http://dx.doi.org/10.1128/JCM.00701-06>). Multiplex PCR was performed on 562 *Salmonella* isolates (as many as 36 isolates per water sample) to predict serotypes. Kauffmann-White serogrouping was used to confirm multiplex PCR pattern groupings before isolates were serotyped, analyzed by pulsed-field gel electrophoresis, and assayed for antimicrobial susceptibility. In 41.2% of the *Salmonella*-positive water samples, all *Salmonella* isolates had identical multiplex PCR patterns; in the remaining 58.8%, two or more multiplex PCR patterns were identified. Within each sample, isolates with matching multiplex PCR patterns had matching serogroups. The multiplex patterns of 495 isolates (88.1%) did not match any previously reported pattern. The remaining 68 isolates matched reported patterns but did not match the serotypes for those patterns. The use of the multiplex PCR allowed the number of isolates requiring further analysis to be reduced to 223. Thirty-three *Salmonella enterica* serotypes were identified; the most frequent included serotypes Muenchen, Rubislaw, Anatum, Gaminara, and IV<sub>50</sub>:z4,z23:–. A majority (141/223) of *Salmonella* isolates clustered into one genotypic group. *Salmonella* isolates in Central Florida surface waters are serotypically, genotypically, and phenotypically (in terms of antimicrobial susceptibility) diverse. While isolates could be grouped as different or potentially the same using multiplex PCR, the multiplex PCR pattern did not predict the *Salmonella* serotype.

*Salmonella* bacteria were traditionally considered zoonotic: they were believed to originate from the gastrointestinal tracts of animals, and outbreaks of salmonellosis were traced back to these reservoirs (1). More recently, the role of the nonhost environment as a source for *Salmonella* bacteria and food contamination has been explored (2–4). One of the nonhost reservoirs of *Salmonella* on which considerable effort has focused is surface water, which poses a potential public health risk through consumption, indirect consumption (for example, irrigation of edible horticultural crops), or direct contact. A number of studies have characterized the distribution of *Salmonella* isolates from different water sources (5–10). In evaluations of surface water for *Salmonella* isolates, multiple serotypes are often identified from a single environmental sample, indicating the diversity of this genus in the environment.

*Salmonella* serotypes isolated from surface waters are often not clinically common (4–12) and often include serotypes of *Salmonella enterica* subsp. *enterica*, *Salmonella enterica* subsp. *arizonae*, and *Salmonella enterica* subsp. *houtenae* (5, 8). Serotypes of *Salmonella enterica* subsp. *enterica* that have been isolated in more than one surface water survey include Anatum, Braenderup, Gaminara, Inverness, Muenchen, Newport, Rubislaw, Saintpaul, and Typhimurium (5–8). The number of different *Salmonella* serotypes isolated from one water sample can differ greatly among samples and may be influenced by the sample volume and enrichment scheme (7, 9); as many as 27 different *Salmonella* serotypes have been reported from a single surface water sampling site (9). The inclusion of multiple watersheds in studies may also lead to the isolation of a large number of different *Salmonella* serotypes (7–9).

A current pitfall of *Salmonella* classification is the length of time it takes to identify serotypes, commonly by serological dis-

crimination with classification according to the Kauffmann-White scheme (13) or through any number of emerging genetic analysis methods (14–18). Many of the rapid methods proposed for *Salmonella* classification are based on, and tested against, the most clinically relevant serotypes (18), and their usefulness in predicting serotypes of environmental isolates is unknown. In order to begin to understand the diversity of *Salmonella* isolates in the nonhost environment, it is crucial to serotype multiple isolates from each sample (9, 10). The use of conventional serotyping for this purpose can be prohibitively expensive, laborious, and time-consuming, making a rapid method desirable. The diversity of 562 *Salmonella* isolates previously recovered from Central Florida surface water sites sampled over 12 months (19) is presented here, along with an evaluation of a previously published multiplex PCR method for rapid serotyping of the isolates (18).

Received 3 July 2014 Accepted 22 August 2014

Published ahead of print 29 August 2014

Editor: C. A. Elkins

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AEM.02191-14>.

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doi:10.1128/AEM.02191-14

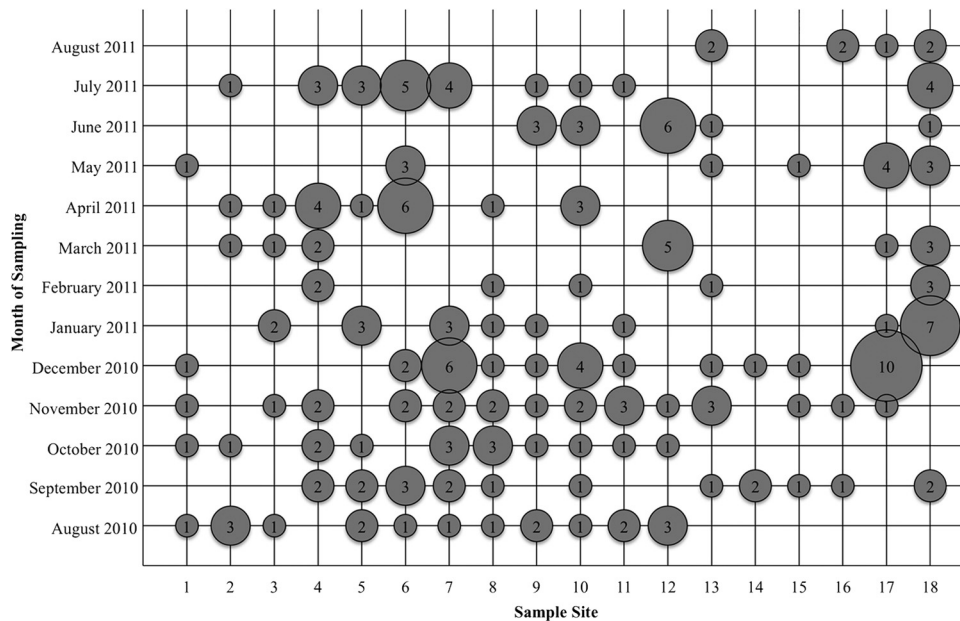


FIG 1 Unique *Salmonella* isolates recovered from 18 sampling sites over the 12-month survey (19). For each combination of sampling site and date, the number of unique isolates collected is given inside a bubble, whose size is relative to that number.

## MATERIALS AND METHODS

**Water sample collection and *Salmonella* isolation.** *Salmonella* isolation from Central Florida surface waters has been described by McEgan et al. (19). Briefly, surface water samples were collected from 18 sites in Central Florida for 12 consecutive months (total, 202 water samples). All sites were in rural agricultural areas, away from animal-agricultural operations, and have been described by McEgan et al. (19). Sampling sites included two lakes, one pond, six creeks, two streams, one river, and six canals. *Salmonella* bacteria were isolated by using a *Salmonella* enrichment procedure modified from that in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) (20). *Salmonella* bacteria were genetically confirmed by PCR of the *invA* and *oriC* genes (21); a total of 562 *Salmonella* isolates were collected (19) and were stored in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 15% glycerol at  $-80^{\circ}\text{C}$ .

**Multiplex PCR analysis.** Multiplex PCR analysis was performed on all *Salmonella* isolates. Frozen ( $-80^{\circ}\text{C}$ ) isolates were streaked onto tryptic soy agar (TSA; Difco, Becton Dickinson) and were incubated for  $24 \pm 2$  h at  $35 \pm 2^{\circ}\text{C}$ . A single colony was then transferred to TSB and was incubated for  $24 \pm 2$  h at  $35 \pm 2^{\circ}\text{C}$ . From these cultures, DNA was extracted using the Mo Bio (Carlsbad, CA, USA) UltraClean DNA kit according to the manufacturer's instructions. Isolated DNA was then subjected to the multiplex PCR described by Kim et al. (18) with minor modifications. The assay uses the amplification of 10 specific gene sections (in two separate PCRs) that result in the production of a serotype-specific pattern of bands to differentiate 30 of the most clinically relevant serotypes of *S. enterica* subsp. *enterica* (18). The presence-absence patterns of the 10 gene sections were compared to the patterns reported by Kim et al. (18). The primers and reaction parameters used here were those described previously by Kim et al. (18), except that all the PCR reagents were obtained from the Fisher exACTGene Complete PCR kit (Fisher Scientific, Pittsburgh, PA, USA). DNA amplicons were separated by agarose (2.5%) gel electrophoresis and were visualized under UV light.

**Serogrouping and serotyping.** Frozen ( $-80^{\circ}\text{C}$ ) isolates were streaked onto TSA and were incubated for  $24 \pm 2$  h at  $35 \pm 2^{\circ}\text{C}$ . A single colony from each isolate was applied to a glass slide with  $5 \mu\text{l}$  sterile saline. *Salmonella* O Poly Antiserum (Becton Dickinson, Sparks, MD, USA) was applied, and autoagglutination was reported after 1 min of rocking of the

glass slide. All isolates were serogrouped in order to determine if isolates that grouped together based on multiplex PCR patterns also belonged to the same serogroup. If isolates from the same water sample had identical presence-absence patterns for the 10 gene sections amplified during the multiplex PCR and belonged to the same serogroup, only one was selected for further analysis. The selected isolates were sent to the National Veterinary Laboratory Service (USDA, Ames, IA, USA) for serotyping.

**PFGE.** Isolates sent for serotyping were analyzed using DNA fingerprinting conducted using XbaI DNA macrorestriction followed by pulsed-field gel electrophoresis (PFGE). PFGE was performed according to the standard laboratory protocol recommended by the Centers for Disease Control and Prevention PulseNet program (22). The PulseNet universal standard strain H9812 of *Salmonella enterica* serotype Braenderup was used as the reference marker.

Gel images were captured using a MultiDoc-It imaging system (UVP, Upland, CA, USA), and images were analyzed using Bionumerics software, version 6.6 (Applied Maths, NV, Austin, TX, USA). Similarity amongst PFGE patterns was assessed using the unweighted-pair group method with arithmetic averages, with 2.0% band position tolerances. Dice coefficients had 1.5% optimization values. PFGE patterns that were  $>80\%$  similar were considered to be in the same genetic cluster; similarity coefficients were obtained by calculating Dice coefficients.

**Antimicrobial susceptibility testing.** Isolates sent for serotyping and analyzed by PFGE were assayed for susceptibility to 15 antibiotics by the calibrated dichotomous sensitivity method (23). The following antibiotics were tested: amikacin (An), amoxicillin-clavulanic acid (Amc), ampicillin (Am), cefoxitin (Fox), ceftriaxone (Cro), cephalothin (Cf), chloramphenicol (C), ciprofloxacin (Cip), gentamicin (G), imipenem (Imp), kanamycin (K), nalidixic acid (Na), streptomycin (S), sulfamethoxazole-trimethoprim (STx), and tetracycline (Te). Antimicrobial susceptibility was determined according to the test standards presented by Bell et al. (23).

## RESULTS

***Salmonella* isolates.** *Salmonella* isolates were collected from 165 (81.7%) of the 202 water samples analyzed (Fig. 1). All *Salmonella* isolates collected displayed typical morphology and growth characteristics on XLT4 agar or *Salmonella* CHROMagar. A total of

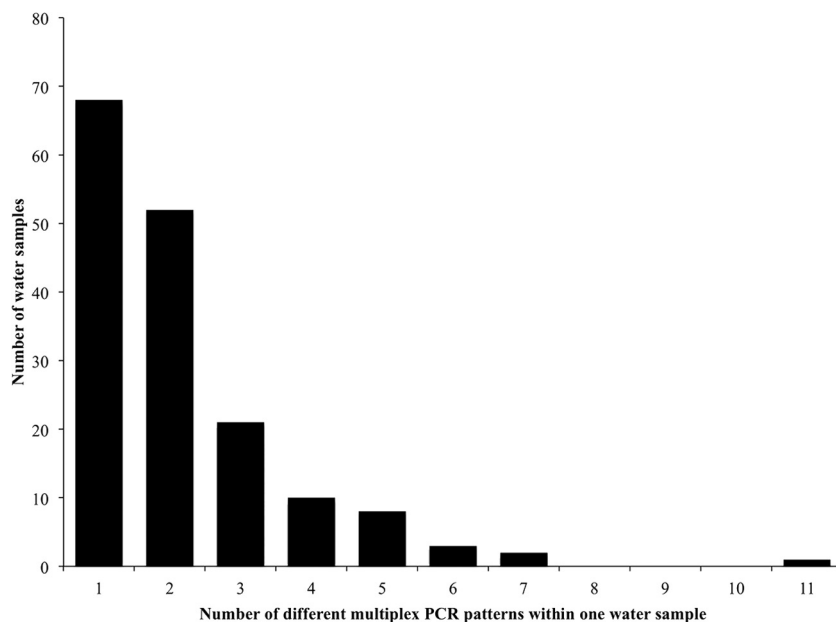


FIG 2 Number of single surface water samples with the indicated number of unique multiplex PCR patterns (18). A single water sample comprises water that was collected from the same site at the same time.

562 isolates were obtained; as many as 36 isolates were obtained and confirmed from a single sample.

**Multiplex PCR, serogrouping, and serotyping.** All 562 isolates were analyzed by multiplex PCR and serogrouping. *Salmonella* isolates from the same sample (water collected from the same site at the same time) with identical presence-absence multiplex PCR patterns were assumed to be potentially the same isolate; *Salmonella* isolates with different multiplex PCR patterns were assumed to be different. In 68 (41.2%) of the 165 water samples from which *Salmonella* isolates were recovered, all *Salmonella* isolates had identical multiplex PCR patterns, as shown in Fig. 2. In 52 (31.5%) water samples, two patterns were identified; in 21 (12.7%) water samples, three patterns were identified among the

isolates. More than three patterns were identified in the remaining 24 (14.5%) water samples.

If a multiplex pattern was previously reported by Kim et al. (18) as a specific serotype, that serotype was designated the “suggested serotype,” and the corresponding serogroup was designated the “suggested serogroup” (Table 1). Sixty-seven of the 562 isolates had multiplex patterns matching those reported by Kim et al. (18), while the multiplex patterns of 495 isolates (88.1%) did not match any pattern reported by Kim et al. For the 67 *S. enterica* isolates matching reported multiplex patterns (18), the suggested serotypes included Bovismorbificans (22 isolates), Braenderup (9 isolates), Chester (1 isolate), Derby (1 isolate), Hadar (3 isolates), Muenchen (4 isolates), Newport (2 isolates), Ohio (9 isolates),

TABLE 1 Suggested serotypes and corresponding serogroups of *Salmonella* isolates recovered from Central Florida surface waters compared with actual serogroups<sup>a</sup>

Suggested serotype	Suggested serogroup	No. of isolates	No. of isolates with the following actual serogroup:							% matching <sup>b</sup>
			A	B	C	D	E	F	G	
Bovismorbificans	B	22	13	5				2		23
Braenderup	B	9		7				1	1	78
Chester	A	1			1					0
Derby	A	1		1						0
Hadar	B	3	1					2		0
Muenchen	B	4	2	2						50
Newport	B	2	2							0
Ohio	B	9		7				1	1	78
Oranienburg	B	4	2	2						50
Poona	B	3	1		2					0
Saintpaul	A	3	2	1						67
Thompson	B	5		5						100
Typhimurium	A	1	1							100
Other		494	57	88	46	6	9	5	17	

<sup>a</sup> Serotypes were suggested on the basis of multiplex PCR patterns corresponding to those reported by Kim et al. (18). The actual serogroups of the isolates were determined by autoagglutination.

<sup>b</sup> Percentage of isolates for which the actual serogroup matched the suggested serogroup.

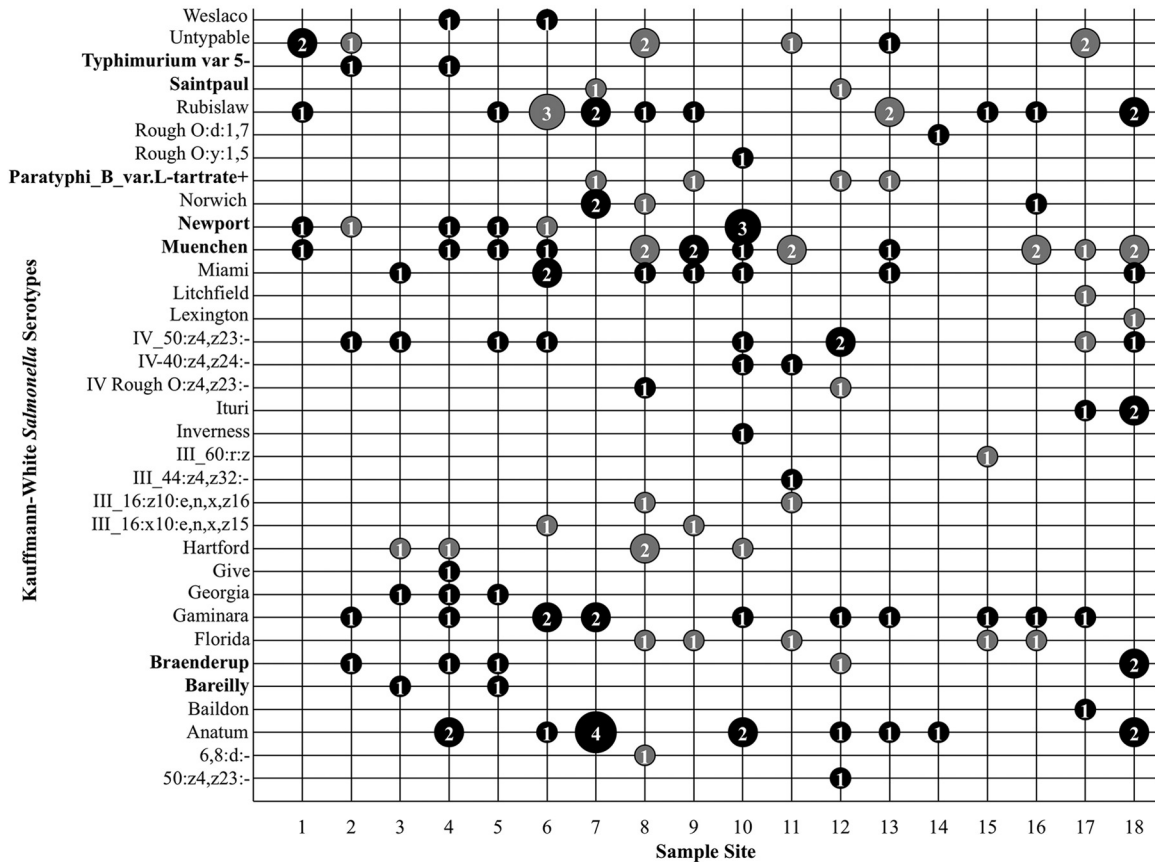


FIG 3 Frequency of isolation of each serotype by location across all 12 sample times. For each combination of sampling site and serotype, the number of unique isolates collected across all sample dates is given inside a bubble, whose size is relative to that number. Black bubbles represent isolates in PFGE cluster A; grey bubbles represent isolates in all other PFGE clusters. Serotypes on the CDC’s list of the 20 most common clinical serotypes are shown in boldface on the y axis.

Oranienburg (4 isolates), Poona (3 isolates), Saintpaul (3 isolates), Thompson (5 isolates), and Typhimurium (1 isolate).

Serogroups were determined for all 562 *S. enterica* isolates and are reported in Table 1 as “actual serogroups.” In all 165 water samples from which *Salmonella* isolates were recovered, if isolates had matching multiplex patterns, as described above, the same isolates also had matching serogroups. However, not all isolates with matching serogroups had identical multiplex PCR patterns within one water sample. Only 31 of the 67 suggested serotypes (46.3%) based on the study of Kim et al. (18) matched the actual serogroup (Table 1).

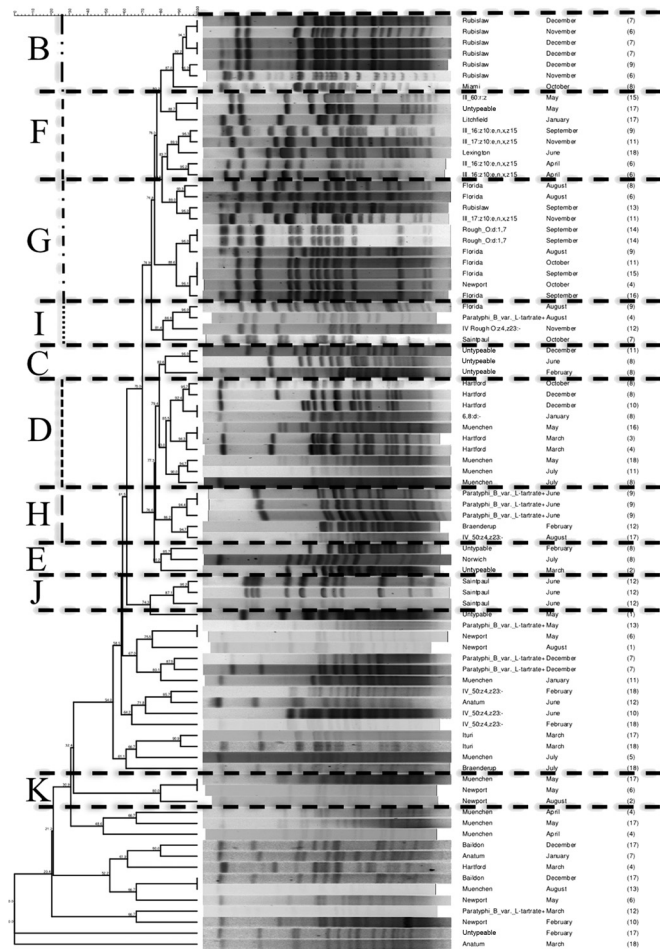
To explore the possibility that the multiplex PCR could be an effective rapid method for determining serotypes of *Salmonella* strains collected from environmental sources, one representative *Salmonella* isolate from each water sample–multiplex PCR pattern combination was sent for serotyping. Of the 562 *Salmonella* isolates analyzed by multiplex PCR and serogrouping, 223 were sent for Kauffmann-White serotyping. Thirty-one *Salmonella* isolates were not recovered following frozen storage and could not be analyzed further. None of the “suggested serotypes” based on the multiplex PCR patterns of Kim et al. (18) matched the serotype determined by Kauffmann-White serotyping.

Kauffmann-White serotyping identified a total of 33 different serotypes for the 223 isolates sent for serotyping. Water samples were collected over a continuous 12-month time frame; the num-

ber of unique isolates from each water sample at each sampling time is given in Fig. 1. The number of different serotypes isolated from any individual sampling site over the course of the 12-month study ranged from 2 at site 14 to 10 at sites 8 and 10. The average number of serotypes isolated from a site was 6. The frequency of isolation for each serotype at each location across all sample times is shown in Fig. 3. No one serotype was isolated in all months of the study from any of the sampling sites. The most frequently isolated *S. enterica* serotypes were Muenchen (11.5% of isolates), Rubislaw (9.5%), Anatum (8.8%), Gaminara (8.8%), and IV\_50:z4,z23:- (6.8%). When the 33 *S. enterica* serotypes isolated in this study were compared to the CDC’s list of the 20 most common clinical serotypes, only 7 were found to be clinically common: serotypes Bareilly, Braenderup, Muenchen, Newport, Paratyphi B var. L-tartrate+, Saintpaul, and Typhimurium var. 5- (Fig. 3) (12). *Salmonella* serotypes isolated during the most months included serotypes Anatum (8 of 12 months), Muenchen (7 of 12 months), and Rubislaw (6 of 12 months). No temporal pattern of *Salmonella* serotype isolation was observed.

**PFGE analysis.** Isolates sent for Kauffmann-White serotyping ( $n = 223$ ) were further analyzed using PFGE–XbaI macrorestriction banding patterns. Two isolates did not produce banding patterns by PFGE, even with the addition of thiourea: a *Salmonella enterica* serotype Bareilly strain, isolated from site 5 in August 2010, which also failed to produce a banding pattern by multiplex





**FIG 5** Dendrogram of *Salmonella* isolates recovered from Central Florida that are not grouped within cluster A, determined by PFGE-XbaI fingerprinting. All isolates that are >80% similar by PFGE are grouped into clusters. Clusters are labeled with letters from B to K, as determined by the complete dendrogram produced from all *Salmonella* isolates recovered from Central Florida (see Fig. S1 in the supplemental material). Lines connecting isolates are as follows: for group B, two short dashes followed by a long dash; for group D, all short dashes; for group F, all medium dashes; for group G, alternating short and medium dashes; for group H, long dashes; for group I, a dotted line. (Only groups with four or more isolates are labeled on the dendrogram.) Additional information on nodal numbers can be found in Fig. S1 in the supplemental material.

*monella* isolates were tested for antimicrobial susceptibility. Twenty-four isolates were susceptible to all antimicrobials tested; 41 were resistant to streptomycin; and 50 were resistant to both streptomycin and kanamycin. Eighteen *Salmonella* isolates were resistant to more than five of the antibiotics tested; the antibiotic resistance profiles of these strains are shown in Table 2. None of the multidrug-resistant (MDR) *Salmonella* isolates were resistant to ceftriaxone or ciprofloxacin. Three of the 13 *Salmonella* Braenderup strains isolated and 3 of the 8 *Salmonella enterica* serotype Florida strains isolated were MDR. The *Salmonella* Braenderup isolates were recovered from three sampling sites (sites 2, 4, and 5) in July and were 87.6% similar by PFGE; the *Salmonella* Florida strains were isolated from sampling sites 4, 5, and 9 during August and October and were 80.8% similar by PFGE. Two isolates each of *Salmonella* Bareilly (of 4 total isolates), *Salmonella* Gaminara

**TABLE 2** Antibiotic resistance profiles of *Salmonella* isolates from Central Florida surface waters<sup>a</sup>

MDR isolate	Site	Month	Antimicrobial resistance profile
Anatum	13	February	Amc Am Fox Cf S Te
Bareilly	4	August	Amc Am Fox Cf K S STx Te
Bareilly	5	August	Amc Am Fox Cf C K Na S STx Te
Braenderup	2	July	Amc Am Cf Imp K S
Braenderup	4	July	Amc Am Fox K Na S STx
Braenderup	5	July	Amc Am Fox Cf C K Na STx Te
Florida	4	August	Amc Am Fox Cf Imp K S
Florida	5	October	Amc Am Fox Cf K S
Florida	9	August	Amc Am Fox Cf K S
Gaminara	15	November	Amc Am Fox Cf C K Na S STx Te
Gaminara	16	November	Amc Am Fox Cf K S
Hartford	3	March	Amc Fox Cf K Na STe
Hartford	4	March	Amc Am Fox Cf C K Na STe
Muenchen	5	July	Amc Cf C K Na STx Te
Muenchen	11	July	Amc Am Cf C K Na STx Te
Rough_O:d:1,7	14	September	Amc Am Fox Cf Gm S
Rubislaw	7	July	Am Fox Cf K S STx Te
Saintpaul	12	June	Amc Am Fox Cf K S

<sup>a</sup> Only isolates resistant to more than five of the antibiotics screened are listed.

(of 13 total isolates), *Salmonella enterica* serotype Hartford (of 5 total isolates), and *Salmonella* Muenchen (of 17 total isolates) were MDR. The *Salmonella* Bareilly strains were isolated from sampling sites 4 and 5 during August 2010 and had identical PFGE patterns; the *Salmonella* Gaminara strains were isolated from sampling sites 15 and 16 during November 2010 and also had identical PFGE patterns. The *Salmonella* Hartford strains were isolated from sampling sites 3 and 4 during March, and the *Salmonella* Muenchen strains were isolated from sampling sites 5 and 11 during July 2011; these isolates had different PFGE patterns. Of the 18 MDR *Salmonella* isolates, 4 isolates were from sampling sites 4 and 5; the remaining sampling sites had only 1 or no MDR *Salmonella* isolates.

**DISCUSSION**

Studies evaluating the prevalence of *Salmonella* bacteria in surface waters have reported different prevalences: 81.7% (165/202) in Central Florida (19), 96% (106/110) (1,000-ml samples) in North Florida (7), 79% (57/72) (555-ml samples) in Georgia (5), 62% (90/145) (Moore swabs suspended in the water for 3 to 5 days) in Ontario, Canada (9), 57% (42/74) (25-ml samples) in North Carolina (6), 11% (16/145) (100-ml samples) in New York State (8), and 7% (18/252) (100-ml samples or 3-day Moore swabs) in California (24). Even within one study of Central California coastal waterways, *Salmonella* prevalences ranged from 0 to 72% in 3,330-ml water samples (25). Differences in water sampling volumes and enrichment protocols complicate a direct comparison of the *Salmonella* prevalence reported here with those reported previously for other regions. To overcome this difficulty, Strawn et al. (10) isolated *Salmonella* strains from the produce environment (including 250-ml water samples) in two locations using identical sample collection, detection, and isolation schemes; *Salmonella* bacteria were isolated from 38% (15/40) of surface water samples in South Florida and 10% of surface water samples (36/354; including those reported in reference 8) in New York State. While it is unfortunate that the numbers of water samples (40 in

South Florida versus 354 in New York State) are not the same, it is clear that differences in *Salmonella* prevalence exist on a regional level, independently of sample size or detection and isolation schemes. These differences may be due to other climatic factors, environmental pressures, wildlife, or agricultural practices. Future studies designed to specifically determine the exact factors that influence pathogen prevalence in surface waters in different regions are warranted.

As has been reported previously (5, 9, 10), more than one *Salmonella* serotype is frequently isolated from a single *Salmonella*-positive surface water sample. Considering only differences in Kauffmann-White serotyping, multiple serotypes were identified in 32/165 *Salmonella*-positive water samples. When multiple *Salmonella* serotypes were identified from a single water sample, the typical number of serotypes identified was 2; as many as 4 different serotypes were isolated from a single water sample. Similarly, of 72 water samples from Georgia, 15 samples yielded 2 serotypes each and 3 samples yielded 3 serotypes each (5). Strawn et al. (10) reported 13/97 water samples with multiple serotypes and 1 sample from South Florida with 3 different serotypes. The different numbers of isolates per sample or isolation scheme selected for further analysis complicate a direct comparison of the numbers of *Salmonella* serotypes from a single water sample. The potential for isolating multiple *Salmonella* serotypes from a single sample highlights the importance of using multiple enrichment and plating media to truly explore *Salmonella* diversity and to facilitate the identification of isolates with atypical characteristics (7, 9, 10).

As few as 7 (New York State) (8) and as many as 38 (Ontario, Canada) (9) different serotypes have been isolated during surface water surveys, indicating a tremendous diversity of *Salmonella* serotypes in the environment. Strawn et al., comparing *Salmonella* isolates from water samples in South Florida and New York State using the same isolation scheme, identified 9 and 12 Kaufmann-White serotypes, respectively, with 4 serovars found in water samples from both locations (10). Surface water surveys in the southeastern United States have reported 5, 8, and 13 different serotypes from North Carolina, North Florida, and Georgia surface waters, respectively (5–7). In addition to sample size and isolation differences, the diversity of serotypes reported here (33 serotypes) may reflect the study design (a greater number of sampling locations and watersheds sampled) or other environmental pressures (wildlife, agricultural practices, etc.).

Of the 33 Kauffmann-White serotypes of *S. enterica* isolated from Central Florida, 15 have been reported in other surface water studies (5–10). They include serotype Anatum (from Georgia and Ontario, Canada), serotype Baildon (from South Florida), serotype Bareilly (from Georgia), serotype Braenderup (from Georgia, North Florida, and South Florida), serotype Gaminara (from Georgia, North Carolina, and South Florida), serotype Give (from Ontario, Canada, and New York State), serotype Hartford (from Ontario, Canada), serotype Inverness (from North Carolina and North Florida), serotype Litchfield (from Ontario, Canada, and South Florida), serotype Miami (from North Carolina), serotype Muenchen (from Georgia, North Florida, and Ontario, Canada), serotype Newport (from North Carolina, North Florida, New York State, Ontario, Canada, and South Florida), serotype Rubislaw (from Georgia, North Florida, New York State, and South Florida), serotype Saintpaul (from Georgia and Ontario, Canada), and serotype Typhimurium (from New York State and Ontario, Canada). None of the 16 serotypes, determined by mul-

tilocus sequencing, from Central California coastal waterways (25) match serotypes in this study. Independently of differences in sampling volume and enrichment protocols, 15/33 serotypes recovered here were also recovered during previous surveys of surface water, suggesting more-frequent presence or greater fitness in surface waters for these serotypes than for other *Salmonella* serotypes. Of the 11 *Salmonella* serotypes isolated in Central Florida and from at least two other locations, only 5 (serotypes Braenderup, Muenchen, Rubislaw, Saintpaul, and Typhimurium) are also reported on the CDC's list of top 20 clinical isolates (12). The frequency, over multiple surface water surveys, of *Salmonella* serotypes that are not often linked to human cases of salmonellosis suggests that the diversity of *Salmonella* strains in the environment includes many serotypes not commonly seen or clinically relevant. Future studies to evaluate potential differences in environmental fitness, associations with hosts, and virulence among surface water *Salmonella* isolates from different regions are justified.

The number of *Salmonella* serotypes differed significantly among the 18 sites tested. The smallest number of serotypes isolated from a single site over the 12 months of our study was 2 from site 14, a canal in a 55% agricultural land usage watershed (19). Two streams and one creek in watersheds with 32 to 33% agriculture (sites 10, 4, and 6) had the highest numbers of serotypes (11, 10, and 9 serotypes, respectively) isolated over the 12 months. *Salmonella* serotypes Anatum, Gaminara, Muenchen, and Newport were common to sites 10, 6, and 4 and were also reported in North Florida (7) and South Florida (10) surface waters. The repeated isolation of these serotypes from Florida surface waters may reflect increased fitness in this environment.

The multiplex PCR described by Kim et al. (18) could not serotype *Salmonella* isolates from Central Florida surface waters. Jean-Gilles Beaubrun et al. (26) report contradictory results, where 76.2% (48/63) of environmental *Salmonella* isolates from tomato farms in Virginia were correctly identified by the multiplex PCR (18) as *S. Newport*, *S. Braenderup*, and *S. Typhimurium*. However, different patterns were reported for *S. enterica* serotypes Berta, Derby, Hadar, Infantis, Montevideo, Muenchen, Newport, and Paratyphi, and more than one multiplex pattern was reported for serotypes Senftenberg and Tennessee (26). While the multiplex PCR (18), did not predict the serotype of *Salmonella* isolates here, the use of multiplex patterns to group isolates collected from one water sample decreased the number of isolates for further characterization by more than half, decreasing the cost and time required to screen the numerous isolates. The use of the multiplex patterns (18) to group isolates introduces the risk of identifying isolates as identical when they are in fact different serotypes, while allowing a greater number of isolates to be screened in a timely manner.

PFGE analysis revealed 19 sets of *Salmonella* isolates with identical banding patterns from multiple months or sampling sites. *Salmonella* Anatum isolates with identical patterns were isolated from sampling site 7 in November, December, and January; the 3-month isolation of *Salmonella* Anatum from one sample site may indicate its survival in the surface water or repetitive introduction from the same source. Repeated surface water samples in which *Salmonella* Anatum isolates with identical PFGE patterns were identified have been seen previously in North Carolina, in an area with a known influence from swine agriculture (6). Repeated isolation of *Salmonella enterica* serotype Heidelberg strains with

identical PFGE patterns from stream water sampled from May through October has been reported in Ontario, Canada (9). *Salmonella* isolates subjected to genotypic clustering by PFGE using only one enzyme (XbaI), as performed here and by Patchanee et al. (9), may be further differentiated by repeating PFGE using a second enzyme or by alternate analysis. As such, the matches described here may be an artifact of the method of analysis selected, supported by the occurrence of more than one multiplex PCR pattern for isolates with identical PFGE patterns.

The majority (115/223) of Central Florida isolates were susceptible to all antimicrobials tested or were resistant only to kanamycin and/or streptomycin, both of which are secondary metabolites of *Streptomyces* spp., common soil microbes that can be found in the environment; this type of resistance, therefore, is not uncommon among environmental isolates (27, 28). Previously, *Salmonella* isolates from watersheds influenced by pig farms had increased antimicrobial resistance (6). Here, sites 4 and 5 had the greatest number of MDR *Salmonella* isolates; while sampling sites selected in this study were not immediately adjacent to animal agriculture, the high prevalence of antimicrobial-resistant isolates may indicate an animal agriculture influence on the watershed.

As in previous studies (5–10, 25), a diverse group of *Salmonella* serotypes was isolated from Central Florida surface waters. Although the isolates could not be serotyped rapidly using the multiplex PCR (18), this technique allowed isolates from the same sample to be grouped as different or potentially the same, decreasing the effort and expense of subsequent typing. However, while the diversity of isolates was also reflected in the genetic profile determined by PFGE using XbaI, the occurrence of more than one multiplex PCR pattern for isolates with identical PFGE patterns indicates the need for more-discriminatory typing tools.

## ACKNOWLEDGMENTS

We thank the USDA CSREES (Specialty Crops Research Initiative 2008-51180-04846) and the University of Florida Alumni Fellowships Fund for financial support of this project.

We thank Gwen Lundy, Luis Martinez, and Brian Buzzie for technical assistance.

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