Applied and Environmental Microbiology, Mar. 2011, p. 1783–1796 Vol. 77, No. 5
0099-2240/11/$12.00 doi:10.1128/AEM.01885-10
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Diversity of Multidrug-Resistant *Salmonella enterica* Strains Associated with Cattle at Harvest in the United States[▽]

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Received 6 August 2010/Accepted 2 January 2011

The prevalence and diversity of multidrug-resistant (MDR) *Salmonella enterica* strains associated with cattle at harvest in the United States were examined. Hides and carcasses of cattle were sampled at processing plants ($n = 6$) located in four geographically distant regions from July 2005 to April 2006. The mean prevalences of *Salmonella* on hides, preevisceration carcasses (immediately after hide removal), and postintervention carcasses (in the chiller and after the full complement of interventions) were 89.6%, 50.2%, and 0.8%, respectively. The values for MDR *Salmonella enterica* strains (defined as those resistant to two or more antimicrobials) as percentages of *Salmonella* prevalence were 16.7% (95% confidence interval [CI], 8.3 to 25.1%; median percent prevalence, 6.9%), 11.7% (95% CI, 4.4 to 19.0%; median, 4.8%), and 0.33% (95% CI, −0.3 to 0.70%; median, 0%), respectively. In this study, 16,218 *Salmonella* hide and carcass isolates were screened for antimicrobial resistance. Of these, 978 (6.0%) unique MDR *S. enterica* isolates were identified and serotyped and their XbaI pulsed-field gel electrophoresis (PFGE) profiles determined. The predominant MDR *S. enterica* serotypes observed were Newport (53.1%), Typhimurium (16.6%), and Uganda (10.9%). Differences in MDR *S. enterica* prevalence were detected, and PFGE analysis revealed both epidemic clusters (profiles found in plants in multiple regions/seasons) and endemic clusters (profiles observed in plants in limited regions/seasons) within several of the MDR serotypes examined. Despite these differences, multiple-hurdle processing interventions employed at all plants were found to be quite effective and decreased *Salmonella* carcass contamination by 98.4% (95% CI, 97.6 to 99.7%).

Salmonellae are important food-borne pathogens noted for causing millions of cases of food-borne illness in the United States each year (26, 55, 66). Nontyphoidal salmonellosis is generally a self-limiting disease, and patients frequently recover without the need for medical attention. However, a small percentage of *Salmonella* infections result in invasive salmonellosis, a more severe form of illness requiring hospitalization and antibiotic therapy. Recent studies have found that in certain *Enterobacteriaceae*, including *Salmonella*, virulence genes may be colocalizing on transferable resistance plasmids (37, 73), a phenomenon that would lend credence to studies that have shown that antimicrobial-resistant *Salmonella* strains may be more invasive than *Salmonella* strains that are susceptible to antimicrobials (45, 71, 72). As such, there is a need to understand the complex etiology and epidemiology of these food-borne pathogens.

Extensive research aimed at characterizing antimicrobial resistance phenotypes of *Salmonella enterica* from a variety of food and animal sources has revealed that numerous serotypes may harbor multiple antimicrobial resistance determinants (75, 80). These multidrug-resistant (MDR) *S. enterica* strains (defined as strains that are resistant to two or more antimicrobial agents) may carry their resistance determinants on chromosomal locations, on resistance plasmids, or on both (3, 23, 52). Of particular importance to the medical community are resistances to the extended-spectrum cephalosporin ceftriaxone, the drug of choice for treatment of pediatric salmonellosis, and to the quinolone nalidixic acid and the fluoroquinolone ciprofloxacin, which are preferable for treatment of adults (42).

While poultry products and, more recently, contaminated fresh produce are well-established vectors for *S. enterica*, several food-borne disease case studies have shown undercooked ground beef and beef products to be sources of sporadic and outbreak cases of salmonellosis (43, 50, 60, 68, 72). Among the various sources or production systems that supply cattle for beef, the primary source of lean beef for the grinding industry is meat harvested from cull cattle (dairy and beef cull cows and bulls). Cull cattle, especially dairy cattle, have been implicated as a reservoir for antimicrobial-resistant *S. enterica* (4, 35, 41, 70). The presence of these organisms on the hides of cattle at harvest represents a risk to food safety, as they may be transferred to carcasses during the dressing process (6, 9, 15). Once on the carcass, pathogens may enter the food supply if they survive carcass-processing interventions. Thus, in order to gain a better understanding of the risk associated with processing cull cattle and the potential for introducing MDR *S. enterica* into the food chain, it is important to study the extent to which the hides of cattle at harvest are contaminated with these pathogens. To that end, we examined the prevalence of MDR *S. enterica* (here referred to as MDR *Salmonella*) associated with cattle at harvest in plants ($n = 6$) located in four geographically distant regions of the United States over the course

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▽ Published ahead of print on 14 January 2011.

of 10 months. The MDR *Salmonella* strains isolated were serotyped and their antimicrobial susceptibility phenotypes and XbaI pulsed-field gel electrophoresis (PFGE) profiles determined. Collection of these data provided a unique opportunity to observe the diversity of MDR *Salmonella* strains found at cattle harvest establishments over time and revealed the existence of both epidemic and endemic MDR *Salmonella* biotypes.

MATERIALS AND METHODS

Sample collection. Processing plants ($n = 6$) that harvest cull cows, bulls, dairy cattle, and/or fed cattle, located in four geographically distant regions (here designated A to D and located within [although not respectively within] microbiological monitoring regions 2, 3, 5, and 8, as defined by the Beef Industry Food Safety Council [BIFSCo] [14]) of the United States, were sampled every 3 months (July, October, January, and April) in a 10-month period from 2005 to 2006. For cull cattle, samples were collected from four plants (three that processed both cull and fed cattle and one that processed strictly cull cattle), one in each region. Samples, including hide samples, preevisceration carcass samples (sampled after hide removal and prior to any intervention), and postintervention carcass samples (sampled after receiving the full complement of processing interventions [including steam vacuum, lactic acid hot-water wash, and spray chill carcass rinse] and having chilled for no more than 2 h), were collected ($n = 95$ per sample type) on each sample day, with two sample days per season, resulting in a total of 3,040 samples for each sample type. Fed-cattle samples were collected from four plants (three that processed both cull and fed cattle and one that processed strictly fed cattle), one in each region, only in the first sample season (July). Hide and preevisceration carcass samples ($n = 171$ to 207) were collected on one sample day per region, for a total of 755 samples for each sample type. Thus, samples were collected from six plants in all, from one or two plants in each region, on a total of 9 days. Hides and carcasses were tagged prior to sampling, such that samples were matched and collected consecutively, with approximately every fourth carcass on the processing line being sampled. All samples were shipped in coolers with ice packs and were received and processed at the U.S. Meat Animal Research Center (USMARC) within 24 h of collection.

Hide samples were obtained by swabbing approximately 1,000 cm^2 with a sterile sponge (Whirl Pak; Nasco, Ft. Atkinson, WI), prewetted with 20 ml sterile Difco buffered peptone water (BPW; Becton Dickinson, Sparks, MD). Hide samples were collected from the brisket plate region of animals on the line, after stunning and exsanguination, prior to hide removal. Carcass samples were obtained by swabbing approximately 8,000 cm^2 of carcass with 2 sterile sponges (Nasco), each prewetted with 10 ml BPW, as previously described (7).

Culture media and enrichment methods. Hide as well as preevisceration and postintervention carcass sample enrichments were analyzed for the presence of *Salmonella*. Sponge samples were enriched, as previously described (7, 12). Briefly, Difco Trypticase soy broth (TSB; Becton Dickinson) was added to sponge samples at a 1:5 ratio, incubated at 25°C for 2 h and 42°C for 6 h, and then held at 4°C until being processed the next day. *Salmonella* strains were isolated from culture enrichments using immunomagnetic separation (IMS), as previously described (56). IMS bead-bacterium complexes were placed into 3 ml of Rappaport-Vassiliadis soya peptone broth (RVS; Oxoid, Basingstoke, United Kingdom) and incubated at 42°C for 18 to 20 h. These secondary enrichments were swabbed onto Difco Hektoen enteric medium (Becton Dickinson) with novobiocin at a concentration of 5 mg $liter^{-1}$ (HEn) and Difco Brilliant Green agar with sulfadiazine at 80 mg $liter^{-1}$ (BGS; Becton Dickinson) and then streaked for isolation and incubated at 37°C for 18 to 20 h. One to three putative *Salmonella* isolates were picked (depending on the number of putative isolates present on the selective medium) and further confirmed as being *Salmonella* by PCR for the *Salmonella*-specific portion of the *invA* gene (58, 62).

Identification and characterization of multidrug-resistant *Salmonella* strains. All *Salmonella* isolates were initially screened for resistance to three antimicrobials (ampicillin [Ap], tetracycline [Te], and kanamycin [K]) by stamping the isolates in a 96-well block format with a Boekel microplate replicator (Boekel Scientific, Feasterville, PA) onto four tryptic soy agar (TSA) plates (150 mm) containing either no antimicrobials, Ap (32 mg $liter^{-1}$), Te (32 mg $liter^{-1}$), or K (64 mg $liter^{-1}$) (all antimicrobials were obtained from Sigma, St. Louis, MO). These plates were incubated at 37°C for 18 to 20 h. Isolates demonstrating resistance to any of these antimicrobials were streaked for isolation onto TSA with the appropriate antimicrobial and were again incubated at 37°C for 18 to 20 h. If multiple isolates from a sample (up to three) demonstrated the same initial resistance pattern in the prescreening process, then one isolate was chosen for further characterization, as previous studies in our laboratory have shown that this frequently indicates the presence of a dominant *Salmonella* strain in the sample enrichment and further analysis yields identical resistance patterns. Cultures on TSA were subcultured by streaking them for isolation onto XLD_{tnc} agar (xylose lysine desoxycholate medium; Oxoid) with 4.6 ml $liter^{-1}$ tergitol (aka niaproof; Sigma), 15 mg $liter^{-1}$ novobiocin, and 10 mg $liter^{-1}$ cefsulodin and incubated at 37°C for 18 to 20 h. Isolates demonstrating typical *Salmonella* colony morphology on XLD_{tnc} (black colonies with a clear pink outer ring) were streaked for isolation onto TSA and incubated, as described above. The resulting pure cultures were used for antimicrobial susceptibility analysis and serological identification. Antimicrobial susceptibility testing was performed using the Sensititre broth microdilution system (TREK Diagnostic Systems, Toledo, OH) and CMV1AGNF test plates, according to the manufacturer's instructions. The plates determined sensitivity to 15 antimicrobials: amikacin (Ai), Ap, amoxicillin-clavulanic acid (Am), ceftiofur (F), cefoxitin (T), ceftriaxone (Ax), chloramphenicol (C), ciprofloxacin (Cp), gentamicin (G), K, nalidixic acid (N), streptomycin (S), sulfisoxazole (Su), Te, and trimethoprim sulfamethoxazole (Sxt). Antimicrobial sensitivity was determined using a Sensititre AutoReader and the SWIN software package, which uses Clinical and Laboratory Standards Institute (CLSI)-approved MIC breakpoint guidelines for the drugs listed above (19, 75). The following organisms were used as quality control strains in the antimicrobial sensitivity assays: *Pseudomonas aeruginosa* ATCC 27853 (American Type Culture Collection), *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923. *Salmonella* isolates were serogrouped with a Welcollex color serogrouping kit (Remel, Lenexa, KS) and serotyped further using slide agglutination with O-factor antisera and tube agglutination with H-factor antisera (Denka Seiken Co., Ltd., Derbyshire, United Kingdom), according to the manufacturer's instructions.

PFGE analysis. PFGE analysis was performed according to the protocol developed by the Centers for Disease Control and Prevention (CDC) (65). Agarose-embedded DNA was digested with XbaI (New England BioLabs, Beverly, MA). *Salmonella enterica* serotype Braenderup strain H9812 was used as a control and for standardization of gels (48). Banding patterns were inspected by visual confirmation and then further analyzed and compared using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium), employing the Dice similarity coefficient with a 1.5% band position tolerance in conjunction with the unweighted-pair group method using arithmetic averages for clustering. For each serotype dendrogram shown, clusters were ranked first by the number of regions and seasons in which isolates within that cluster were observed and then by the percentage of isolates that were present within each cluster. Roman numerals were used for cluster designations, and in each case, Roman numeral I indicates the cluster that was found to be the most widely distributed. Clusters were designated sequentially from there, based on the percentage of isolates that fell within that cluster.

Statistics. Cull cattle *Salmonella* prevalence data were analyzed by season (8 sample days per season; 2 days at each of four plants) or by region (8 sample days per plant in each region; 2 days per season). Fed-cattle prevalence values are reported as percent positive samples for one sample collection day per plant in each region. Prevalence values for cull and fed-cattle samples were calculated by dividing the number of culture-positive samples by the total number of samples collected (cull cattle $n = 95$ per sample type per day; fed cattle $n = 171$ to 207 per sample type). The percents prevalence for *Salmonella* and MDR *Salmonella* on hide and carcass samples were calculated for each sample day and are reported as the mean percent prevalence, 95% confidence interval (95% CI), and median percent prevalence values (Tables 1 and 2). For data sets that were not normally distributed, comparisons of median prevalence values were made using the Kruskal-Wallis test for nonparametric data and Dunn's multiple-comparison posttest. For data sets that were normally distributed, comparisons of mean prevalence values were made using a one-way analysis of variance (ANOVA) and Bonferroni's multiple-comparison posttest. Data were analyzed using Prism 5.0 Graph Pad software, and P values less than 0.05 were considered significant.

The diversity of MDR *Salmonella* serotypes isolated from hide samples was examined by calculating Simpson's index of diversity (ID) ($1 - D$, where D is $\Sigma[n(n-1)]/N(N-1)$, with n representing the number of unique isolates of each serotype of MDR *Salmonella* identified on a given sample day and N representing the total number of unique MDR *Salmonella* isolates identified on a given sample day). Simpson's index of diversity is a measure of both how many MDR serotypes are isolated in each region on a given sample day and how evenly those serotypes are represented in a given region or season. Values for $1 - D$ that are closer to 1 indicate high diversity, while values closer to 0 indicate low diversity. Values were averaged by region or season and reported as mean levels of diversity with the 95% CI of each calculated mean. Comparisons of values for

TABLE 1. Mean percents *Salmonella* and MDR *Salmonella* prevalence by season

Sample group and parameter	Value for:												*P*	
	Summer		Fall		Winter		Spring		Overall					
	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR
Hide (cull)														
% *Salmonella*	78.5	15.3	75.0	13.4	65.3	26.3	74.5	11.6	72.9	16.7	0.4864[a]	0.8803[a]
95% CI	55.6–101.4	−2.6–33.3	55.9–94.1	2.4–24.7	37.1–89.9	−4.8–57.4	56.5–92.4	−1.7–24.9	63.7–82.1	8.3–25.1		
Median % prevalence	91.6	3.2	78.5	7.9	77.4	6.4	76.3	6.3	80.6	6.9		
Simpson's ID		0.41		0.49		0.28		0.39		0.39		0.4828[b]
95% CI		0.20–0.61		0.26–0.74		0.02–0.53		0.12–0.65		0.29–0.49		
Preevisceration (cull)														
% *Salmonella*	39.9	9.5	40.4	11.4	32.2	23.1	41.4	2.8	38.5	11.7	0.8928[b]	0.2336[a]
95% CI	14.3–65.4	1.4–17.5	15.6–65.1	3.2–19.7	10.2–54.1	−7.9–54.2	26.2–56.7	0.51–5.0	29.3–47.7	4.4–19.0		
Median % prevalence	35.3	6.4	35.8	6.4	27.0	4.2	44.2	2.7	37.9	4.8		
Postintervention (cull)														
% *Salmonella*	0.54	0.8	0.0	0.14	0.41	0.26	0.92	0.14	0.47	0.33	0.3284[a]	0.8466[a]
95% CI	−0.42–1.5	−0.75–2.3	0.0	−0.19–0.46	−0.06–0.88	−0.36–0.88	−1.3–3.1	−0.18–0.46	−0.04–0.98	−0.03–0.70		

[a] Comparisons of median prevalence values were made using the Kruskal-Wallis test for nonparametric data and Dunn's multiple-comparison posttest ($P < 0.05$).
[b] Comparisons of mean prevalence or diversity values (normally distributed data sets) were made using a one-way ANOVA and Bonferroni's multiple-comparison posttest ($P < 0.05$).

Simpson's index of diversity were made using a one-way ANOVA and Bonferroni's multiple-comparison posttest (39, 67).

RESULTS

Prevalence of *Salmonella* and MDR *Salmonella* on cattle hide and carcass samples. *Salmonella* mean prevalence values for cull cattle hide samples, preevisceration carcass samples, and postintervention carcass samples were fairly consistent across seasons and on average were 89.6, 50.2, and 0.8%, respectively, as we have reported previously (15). These values are listed in Table 1 and are separated into the percentages of samples in each season that were found to be contaminated with non-MDR *Salmonella* or MDR *Salmonella*. These data show that the proportions of hide and carcass samples that were found to contain MDR *Salmonella* were also fairly consistent across seasons and were on average 16.7, 11.7, and 0.33% (median values of 6.9, 4.8, and 0%) for hides, preevisceration carcass samples, and postintervention carcass samples, respectively (Table 1). In contrast with seasonal prevalence, analysis of *Salmonella* and MDR *Salmonella* prevalence by region revealed significant differences (Table 2). Specifically, while the overall prevalence of *Salmonella* on hides and carcasses of cull cattle sampled at harvest in region C was consistent with that found in other regions, the prevalence of MDR *Salmonella* detected in region C was consistently lower than (albeit not significantly different from) that observed in plants sampled in region A or B. And throughout the course of the study, MDR *Salmonella* prevalence values in region C were significantly lower ($P = 0.0008$) than those observed in region D (Table 2). No significant regional differences were observed for MDR *Salmonella* prevalence values associated with postintervention carcasses.

Salmonella and MDR *Salmonella* prevalence associated with fed cattle was examined in the summer sample season only and was found to be reflective of cull cattle prevalence in the same region (Table 2). Analysis of the MDR *Salmonella* strains isolated from fed cattle (for those harvested in the same plant as the cull cattle sampled in this study) revealed strong evidence of cross-contamination in the plant environment, as indistinguishable *Salmonella* strains (as characterized by serotype, MDR resistance phenotype, and PFGE profile) were frequently collected over consecutive sampling days. This cross-contamination effect has previously been noted by others (5, 34), and as a result, prevalence and MDR serotype/phenotype data collected from fed cattle were treated as an additional sample time point for surveying the diversity of MDR *Salmonella* strains entering slaughter establishments on the hides of cattle. No attempt to attribute any specific serotype or MDR phenotype to one or the other cattle type was made, as the data were not collected in such a way as to substantiate this type of analysis.

Sampling of 3,040 postintervention carcasses over the course of this study resulted in a total of 24 *Salmonella* isolates (10 MDR and 14 pansusceptible isolates) that were collected from 23 postintervention carcasses. Thus, despite the infrequent isolation of *Salmonella* from postintervention carcasses (*Salmonella* prevalence at this sample site was on average 0.8% [95% CI, 0.18 to 1.42%]), the mean percentage of *Salmonella* strains

TABLE 2. Mean percents *Salmonella* and MDR *Salmonella* prevalence by region

Sample group and parameter	Value for:[a]										*P*	
	A		B		C		D		Overall			
	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR	Not MDR	MDR
Hide (cull)												
% *Salmonella*	80.4[C]	7.6[AB]	80.9[C]	11.2[AB]	91.1[C]	1.9[A]	38.9[D]	46.0[B]	72.9	16.7	0.0004[b]	0.0008[b]
95% CI	66.8–93.9	0.5–14.8	70.5–91.4	3.3–19.1	83.6–98.6	–0.3–4.3	19.4–58.4	20.9–71.0	63.7–82.1	8.3–25.1		
Median % prevalence	81.6	3.7	80.0	10.0	94.8	1.6	35.4	43.7	80.6	6.9		
Simpson's ID		0.41		0.5		0.1		0.5		0.4		0.0785[c]
95% CI		0.19–0.64		0.26–0.74		−0.18–0.38		0.33–0.59		0.29–0.49		
Hide (fed)												
% *Salmonella*	81.3	18.7[d]	79.2	2.9	98.4	0.0	69.1	17.6	82.0	9.8		
95% CI									62.6–101.4	−5.7–25.3		
Preevisceration (cull)												
% *Salmonella*	45.8[CD]	6.4[AB]	58.3[D]	8.9[A]	25.6[C]	1.3[B]	24.2[C]	30.1[A]	38.5	11.7	0.0113[c]	0.0026[b]
95% CI	26.6–65.0	−1.1–13.9	35.9–80.6	2.8–15.1	15.0–36.1	−0.29–2.9	4.5–43.9	1.9–58.3	29.3–47.7	4.4–19.0		
Median % prevalence	45.8	2.1	55.2	6.8	26.5	0.0	9.5	18.5	37.9	4.8		
Preevisceration (fed)												
% *Salmonella*	53.8	14.6[d]	34.6	2	60.2	0.0	14.4	4.3	40.8	5.2		
95% CI									7.9–73.6	−5.1–15.6		
Postintervention (cull)												
% *Salmonella*	0.54	0.8	0.27	0.14	0.14	0.0	0.9	0.4	0.5	0.3	0.8791[b]	0.4525[b]
95% CI	−0.42–1.5	−0.75–2.3	−0.15–0.7	−0.19–0.46	−0.19–0.46	0.0	−1.3–3.1	−0.26–1.1	−0.04–0.98	−0.03–0.70		

[a] Common uppercase superscripts (A and B, MDR *Salmonella*, and C and D, non-MDR *Salmonella*) indicate values that are not significantly different ($P < 0.05$).
[b] Comparisons of median prevalence values were made using the Kruskal-Wallis test for nonparametric data and Dunn's multiple-comparison posttest.
[c] Comparisons of mean prevalence or diversity values (normally distributed data sets) were made using a one-way ANOVA and Bonferroni's multiple-comparison posttest.
[d] Fed cattle MDR *Salmonella* prevalence values for hides and carcasses in region A (measured in summer only) were reflective of cull cattle MDR *Salmonella* prevalence values for the same region and season. MDR *Salmonella* prevalence values for cull cattle hides and preevisceration carcasses in this region and season were as follows: for hide samples, 4.2% for day 1 and 20% for day 2, and for preevisceration carcass samples, 2.5% for day 1 and 47.9% for day 2. These results demonstrate the wide range in prevalence values that can be observed from one sample day or time point to the next.

found to be MDR at this site was 43.3% (95% CI, 7.7 to 78.9%) in this study.

***Salmonella* serotype diversity and MDR phenotypes.** Antimicrobial sensitivity screening of 16,218 *Salmonella* isolates revealed 978 (6.0%) unique MDR *Salmonella* isolates. Unique isolates refer to a single isolate per sample except for rare instances (~0.03%; n = 10,630 samples examined) when more than one serotype of MDR *Salmonella* was isolated from an enrichment. Of these isolates, 870 were obtained from cull cattle samples (n = 9,120) and 108 from fed cattle samples (n = 1,510). MDR *Salmonella* strains were isolated from samples collected at five of the six plants that participated in this study, and 59 different resistance phenotypes were observed (Fig. 1). Serotyping of these MDR *Salmonella* strains resulted in the identification of 20 serotypes. The most prevalent MDR *Salmonella enterica* serotype observed was Newport (53.1%), followed by Typhimurium (16.6%) and Uganda (10.9%) (Fig. 1). Other MDR *Salmonella enterica* serotypes identified included Agona (5.9%), Anatum (4.2%), Reading (3.3%), Dublin (1.4%), Muenster (0.8%), Ohio (0.8%), Give (0.6%), Heidelberg (0.6%), Saint Paul (0.4%), Infantis (0.3%), Derby (0.2%), Mbandaka (0.2%), Montevideo (0.2%), Cerro (0.1%), Enteritidis (0.1%), Kentucky (0.1%), and Muenchen (0.1%).

Analysis of MDR *Salmonella* serotype diversity using Simpson's index showed moderate levels of diversity in the sample periods examined. In this study, cattle were sampled consecutively (generally every fourth animal over consecutive lots), and as a result of this sampling scheme, MDR *Salmonella* strains present on cattle hides and carcasses in a given sample period were typically dominated by a particular serotype/MDR phenotype, likely a reflection of cattle lot effects and cross-contamination in the lairage environment (5, 15, 34). Accordingly, mean diversity values were low to moderate and ranged from 0.28 to 0.49 when analyzed by season or from 0.1 to 0.5 when analyzed by region. While not significantly different, seasonal diversity was observed to be lowest in the winter and highest in the fall (P = 0.4828), and analysis by region showed that diversity tended to be lowest in region C and highest in region B (P = 0.0785) (Tables 1 and 2).

MDR *Salmonella* Newport was the most frequently isolated and widely distributed serotype observed in this study. It was isolated at least once from plants in all four regions and in all seasons (Fig. 2). The mean observed prevalence values per sample day ranged from 0.12% to 28.4% and from 0.47% to 21.1% for hide and preevisceration carcass samples, respectively (Fig. 2). MDR *Salmonella* serotypes Agona, Anatum, and Reading were also widely distributed and isolated from cattle at harvest in all four regions, albeit at lower levels than Newport (Fig. 2). While the aforementioned serotypes appeared to be widely distributed, other serotypes identified were found to have a more regional distribution. For example, although MDR *Salmonella* Typhimurium was isolated from cattle at harvest in plants in all four regions, considerably higher prevalence values were observed in plants in regions A and B than in region C or D. This phenomenon also was observed for MDR *Salmonella* serotypes Dublin and Uganda, which were predominantly isolated from cattle at harvest in plants in regions B and D (Fig. 2).

Characterization of the antimicrobial susceptibility profiles for 978 MDR *Salmonella* isolates showed the most common resistance pattern to be resistance to eight antimicrobials (AmApFTCSSuTe), with decreased susceptibility to ceftriaxone (Ax) (MIC range between 16 and 32 μg ml^{-1}) (Fig. 1). Approximately one-third of all MDR *Salmonella* strains isolated in this study (31.3%) demonstrated this resistance pattern. The *Salmonella* serotypes found exhibiting this profile included Newport, Typhimurium, Agona, Anatum, Reading, Dublin, Enteritidis, Mbandaka, Saint Paul, Heidelberg, Give, and Ohio. Resistance to Ax was detected in 12.1% of all MDR *Salmonella* strains examined, while decreased susceptibility to Ax [here indicated by parentheses as "(Ax)"] was detected in 66.9% of the isolates examined. Noteworthy was the low incidence of Ax resistance associated with *Salmonella* serotype Typhimurium (0.6% of MDR *Salmonella* Typhimurium strains were resistant to Ax, while 9% demonstrated decreased susceptibility). Resistance to quinolone and fluoroquinolone antimicrobials was rarely detected, with 0.3% of the MDR *Salmonella* strains showing resistance to nalidixic acid (*Salmonella* serotypes Dublin, Agona, and Uganda), and no isolates were found with resistance to ciprofloxacin. *Salmonella* serotype Uganda was observed to have the most extensive resistance phenotype, with resistance to 12 of the 15 antimicrobials screened (Fig. 1).

The MDR *Salmonella* strains isolated from postintervention carcasses (n = 10) included those of serotypes (MDR phenotypes) Typhimurium (AmApCSSuTe), Dublin [AmApFT(Ax)CGKSSuTe], Reading [AmApFT(Ax)CSSuTe], and Newport [AmApFT(Ax)CKSSuTeSxt] and a nontypeable (NT) O-group D isolate (AmCKSSuTe). The dominant MDR *Salmonella* serotype observed at this sample site was Typhimurium (n = 6), while the other MDR *Salmonella* strains were each isolated once. The pansusceptible (PS) *Salmonella* strains (n = 14) isolated were dominated by serotype Dublin (n = 7). Other PS *Salmonella enterica* serotypes identified included Muenster (n = 2), Anatum (n = 2), and Cerro, Onrieke, and Montevideo (which were each isolated once).

XbaI PFGE analysis. The XbaI PFGE profiles of MDR *Salmonella* strains collected in this study showed that isolates predominantly clustered by serotype and MDR phenotype, a phenomenon previously reported by others (44). Comparisons of XbaI profiles of the MDR *Salmonella* Newport isolates showed the overall similarity to be 79.3%, and the majority of isolates fell into seven PFGE clusters (Fig. 3). Certain clusters (e.g., cluster I) appeared to be widely disseminated and were isolated from cattle at harvest in plants located in multiple regions of the United States over multiple seasons. Conversely, other clusters appeared to be endemic, as they were isolated repeatedly over the course of the study, but primarily from plants in a limited number of regions (e.g., cluster II) (Fig. 3). Comparisons of the XbaI PFGE profiles of the MDR *Salmonella* Typhimurium isolates showed their overall similarity to be 69.3%, and these isolates fell predominantly into two major groups composed of five clusters (Fig. 4). The first group consisted of three clusters and included isolates exhibiting core resistance to ApKSSuTe (cluster III) and isolates with PFGE profiles similar to those of the recently described WA-TYP035/187 MDR clade (1, 31) (clusters IV and V), some of which demonstrated cephalosporin resistance. The second group of isolates exhibited the ApCSSuTe DT104 resistance phenotype (cluster I) or an expanded version of this phenotype with ami-

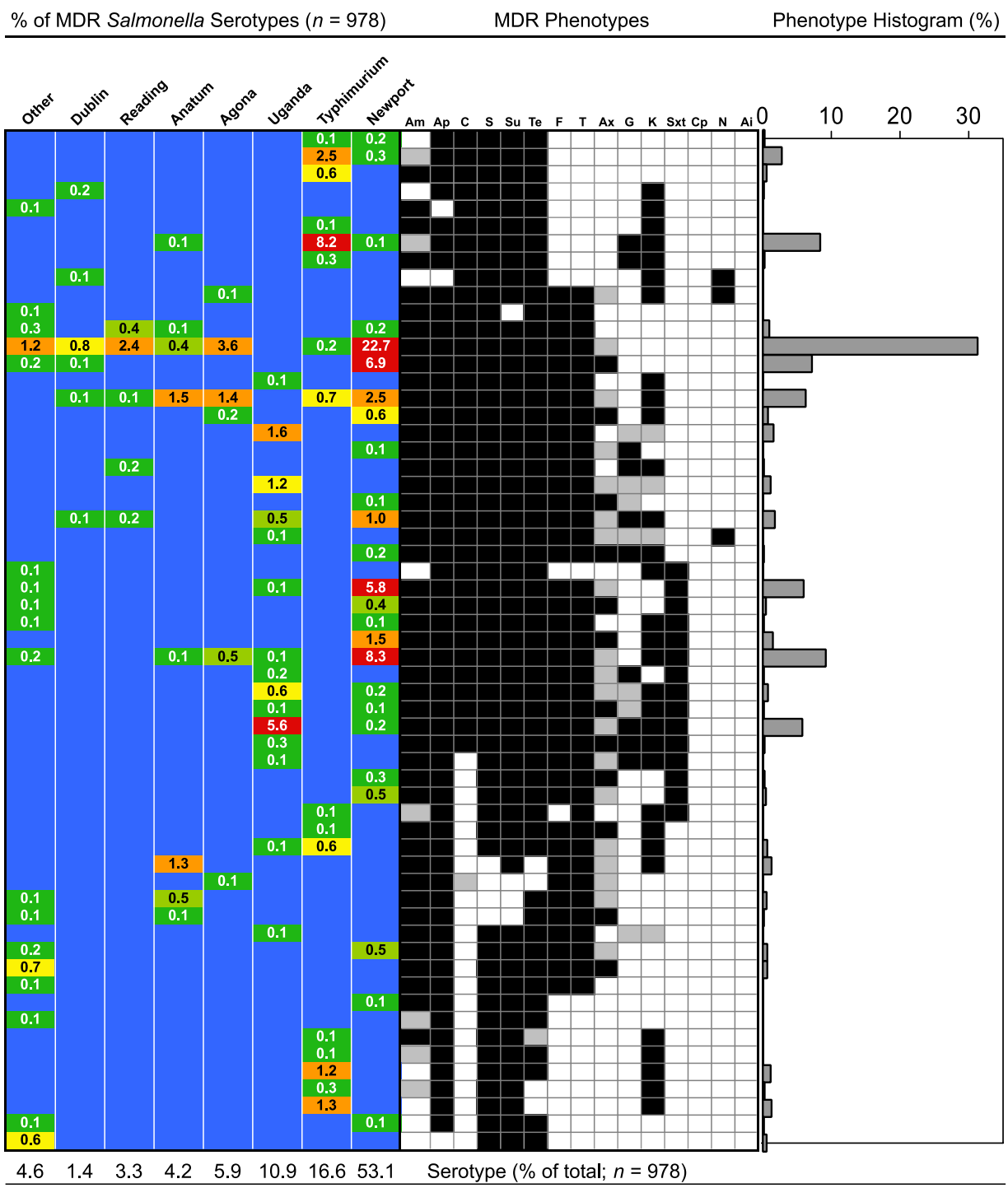

FIG. 1. Serotype distribution heat map, antimicrobial resistance profile, and histogram depicting the percentage of each resistance phenotype observed in the 978 MDR *Salmonella* strains characterized. Values in the heat map indicate the percentage of each serotype that was found to exhibit the corresponding resistance phenotype. Resistance phenotypes are indicated by black (resistant), gray (intermediate), or white (susceptible) boxes. Antimicrobial abbreviations: Am, amoxicillin-clavulanic acid; Ap, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; G, gentamicin; K, kanamycin; Sxt, sulfamethoxazole-trimethoprim; Cp, ciprofloxacin; N, nalidixic acid; and Ai, amikacin.

noglycoside resistance (cluster II). While the majority of MDR *Salmonella* Typhimurium isolates were obtained from plants sampled in regions A and B, isolates from cluster I (exhibiting the classic epidemic DT104 PFGE profile [27, 53]) appeared to be widely disseminated and were found in all regions sampled at various times of the year. In contrast, representatives of clusters III to V were isolated from cattle at harvest in plants only in certain regions (Fig. 4).

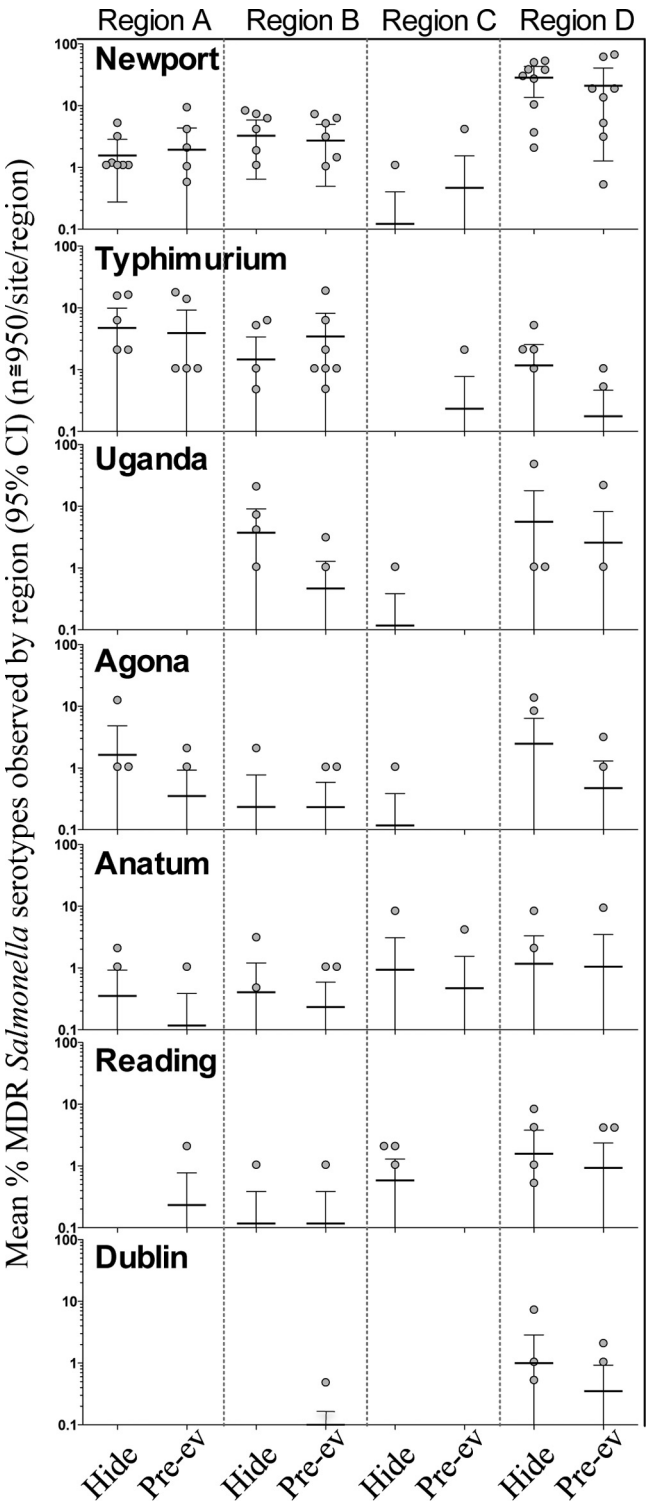

FIG. 2. Top seven MDR *Salmonella* serotypes observed at processing plants in four regions of the United States over 10 months. Each point represents the percent prevalence of that serotype on a given sample day (n = 95 [cull] or 177 to 180 [fed]/sample site; values are shown for hide or preevisceration carcass [pre-ev] samples per sample day; 9 sample days per region). Also depicted are the mean (horizontal lines) and 95% confidence intervals (bars) for the percent prevalence observed for each serotype in each region.

While certain MDR *Salmonella* Newport and Typhimurium clusters could be found widely disseminated, MDR *Salmonella* Uganda and Dublin strains were isolated primarily from cattle at harvest in regions B and D. The PFGE profiles of *Salmonella* Uganda were 79.4% similar overall and fell into two major groups. Isolates in the first group (cluster I) were found primarily in regions B and D, although one of these isolates was collected from region C in the fall (Fig. 5). In two of the four sample seasons, isolates in the second group (cluster II) were found only in region D. The *Salmonella* serotype demonstrating the most highly conserved PFGE profiles was MDR Dublin (87.9% similarity overall), and the isolates fell into three clusters, two of which comprised isolates found only in region D and one of which comprised isolates found in both regions B and D at different times of the year (Fig. 6).

DISCUSSION

Numerous studies have examined the prevalence of *Salmonella* on hides and carcasses of fed and cull cattle at slaughter (9, 11, 15, 39, 64). However, studies examining antimicrobial-resistant-*Salmonella* prevalence and diversity in beef production settings are fewer (9, 51, 78). Research efforts aimed at characterizing MDR *Salmonella* strains associated with feedlot or dairy cattle have primarily focused on fecal shedding of these pathogens from animals in farm settings (2, 29, 30, 35, 36, 74). While studies such as these add to our understanding of the epidemiology of these pathogens, surveys of MDR *Salmonella* prevalence on cattle hides at harvest are perhaps more directly related to addressing the food safety concerns of beef. The reasons for this are 2-fold. First, the ability to detect *Salmonella* in hide samples is likely enhanced in comparison with that ability for fecal samples of asymptomatic cattle. This is probably due to increased target bacterial concentration in the less restrictive, generally aerobic hide environment. Second, and perhaps more important, cattle hides are a major source of carcass contamination in beef processing environments (54, 57). Previous analysis of the aerobic bacterial load (aerobic plate count [APC]) of hide and preevisceration carcass samples collected in this study (n = 3,040 for each sample site) showed that on average ~1.7% (95% CI, 0.96 to 3.13%) of hide contamination was observed to be transferred to carcasses during the dressing process (15). Also, numerous studies have shown that cattle hide hygiene is significantly affected by transportation and lairage prior to slaughter (5, 24, 49, 63). Accordingly, measurements of hide pathogen prevalence at harvest are an essential aspect of both understanding the magnitude of risk and determining what control measures may be necessary to mitigate those risks.

In this study, we found that MDR salmonellae were a consistently measurable subpopulation of the salmonellae present on hides and carcasses of cattle at slaughter. While the majority of *Salmonella* strains isolated were found to be sensitive to ampicillin, tetracycline, and kanamycin (and, in keeping with previous studies in our laboratory, were thus likely susceptible to the other 15 antimicrobials evaluated) or were found to be resistant to 1 antimicrobial (commonly tetracycline), MDR *Salmonella* strains were isolated from 16.7% (95% CI, 8.3 to 25.1%) of hide samples, 11.7% (95% CI, 4.4 to 19.0%) of preevisceration carcass samples, and 0.33% (95%

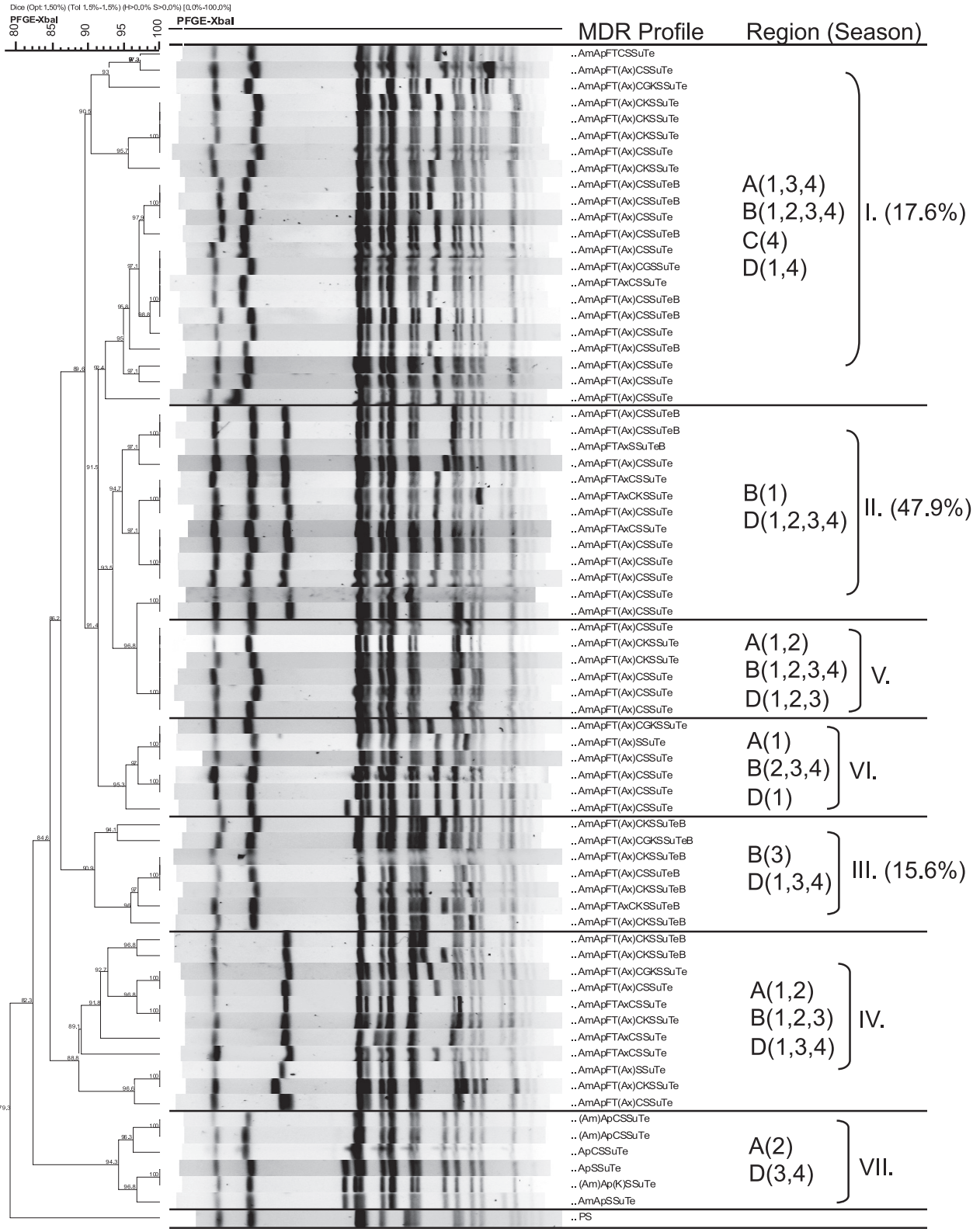

FIG. 3. XbaI PFGE-based dendrogram and MDR profiles of representative *Salmonella* Newport isolates. Cluster analysis of banding patterns was performed using the Dice similarity coefficient and the unweighted-pair group method. Regions (A to D) and seasons (1, summer; 2, fall; 3, winter; 4, spring) where isolates were observed are indicated, in addition to the percentage of *Salmonella* Newport isolates found in that cluster, for the top three clusters. Antimicrobial abbreviations: Am, amoxicillin-clavulanic acid; Ap, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; G, gentamicin; K, kanamycin; Sxt, sulfamethoxazole-trimethoprim; Cp, ciprofloxacin; N, nalidixic acid; and Ai, amikacin.

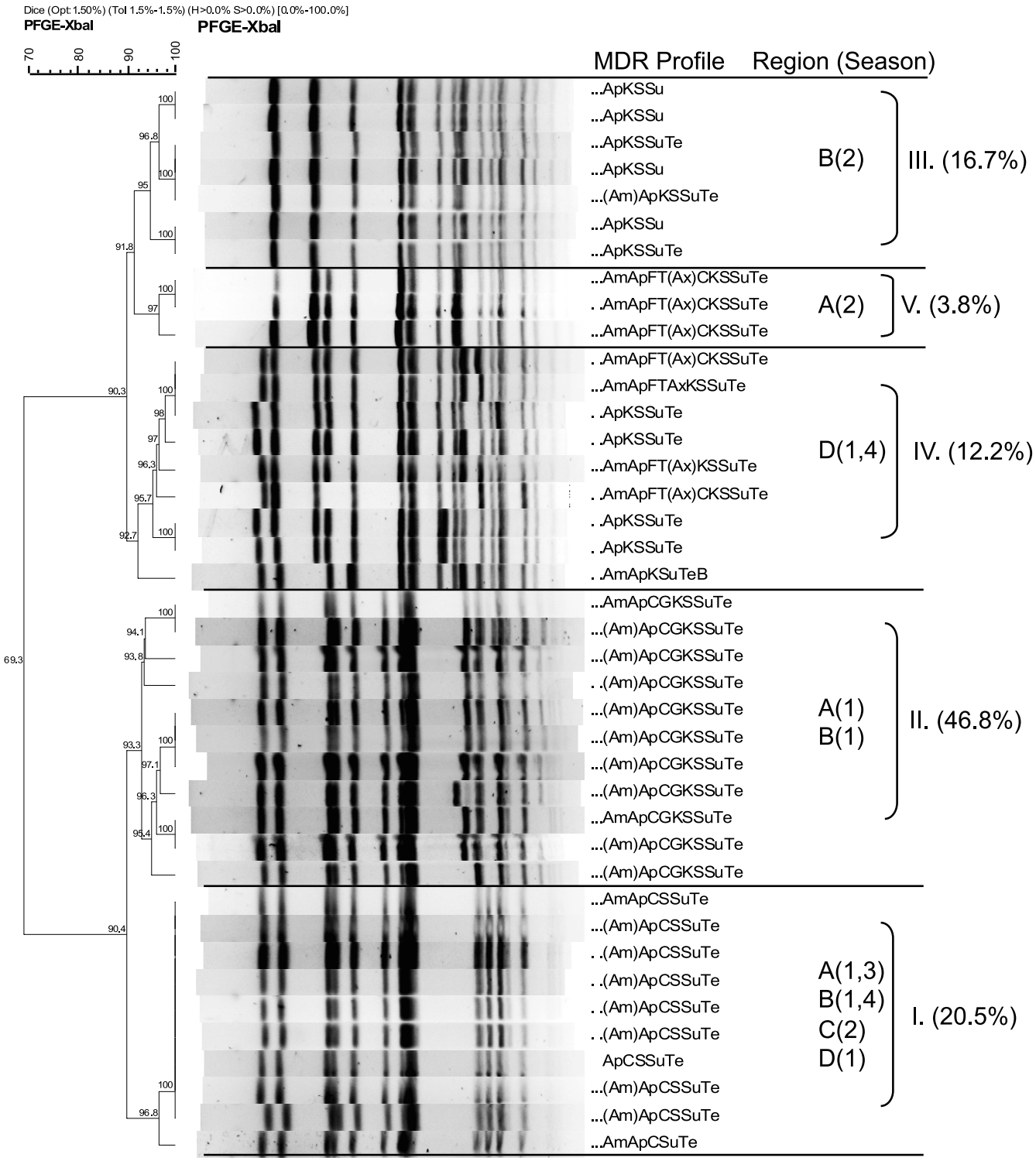

FIG. 4. XbaI PFGE-based dendrogram and MDR profiles of representative *Salmonella* Typhimurium isolates. Cluster analysis of banding patterns was performed using the Dice similarity coefficient and the unweighted-pair group method. Regions (A to D) and seasons (1, summer; 2, fall; 3, winter; 4, spring) where isolates were observed are indicated, in addition to the percentage of *Salmonella* Typhimurium isolates found in that cluster. Antimicrobial abbreviations: Am, amoxicillin-clavulanic acid; Ap, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; G, gentamicin; K, kanamycin; Sxt, sulfamethoxazole-trimethoprim; Cp, ciprofloxacin; N, nalidixic acid; and Ai, amikacin.

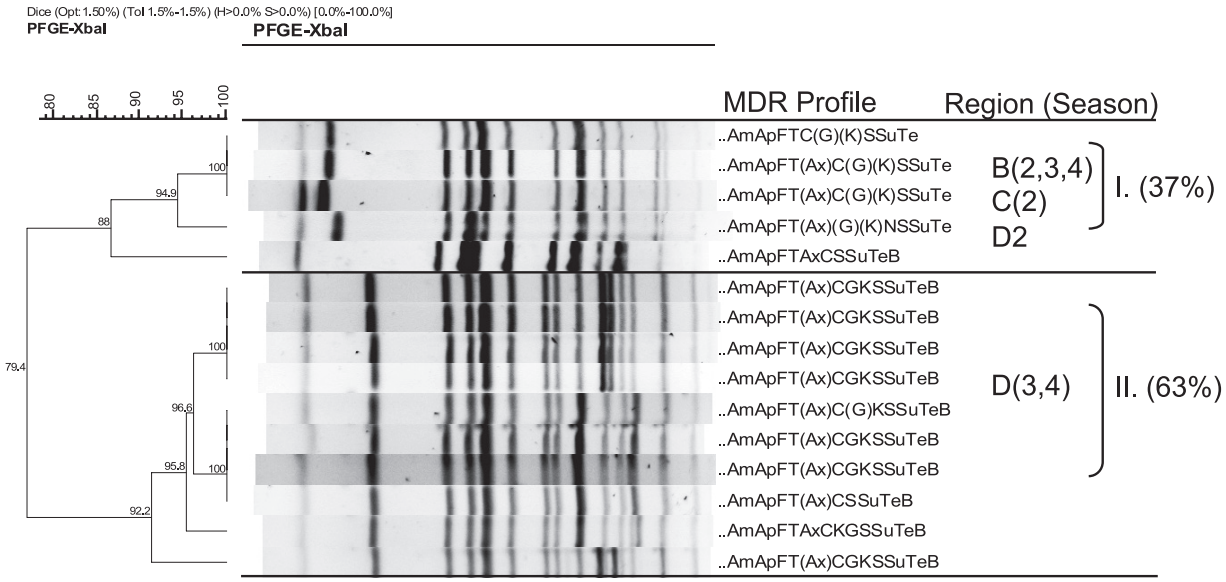

FIG. 5. XbaI PFGE-based dendrogram and MDR profiles of representative *Salmonella* Uganda isolates. Cluster analysis of banding patterns was performed using the Dice similarity coefficient and the unweighted-pair group method. Regions (B to D) and seasons (1, summer; 2, fall; 3, winter; 4, spring) where isolates were observed are indicated, in addition to the percentage of *Salmonella* Uganda isolates found in that cluster. Antimicrobial abbreviations: Am, amoxicillin-clavulanic acid; Ap, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; G, gentamicin; K, kanamycin; Sxt, sulfamethoxazole-trimethoprim; Cp, ciprofloxacin; N, nalidixic acid; and Ai, amikacin.

CI, −0.03 to 0.7%) of postintervention carcass samples on average. The mean prevalence values for hide, preevisceration carcass, and postintervention carcass samples were somewhat greater than the median prevalence values (6.9%, 4.8%, and 0.0%, respectively), likely a reflection of the large differences in MDR *Salmonella* prevalence observed between plants in different regions. Analysis of MDR *Salmonella* prevalence by season showed no significant differences, although analysis by region did. Specifically, MDR *Salmonella* prevalence in plants in region C was consistently (although not significantly) lower

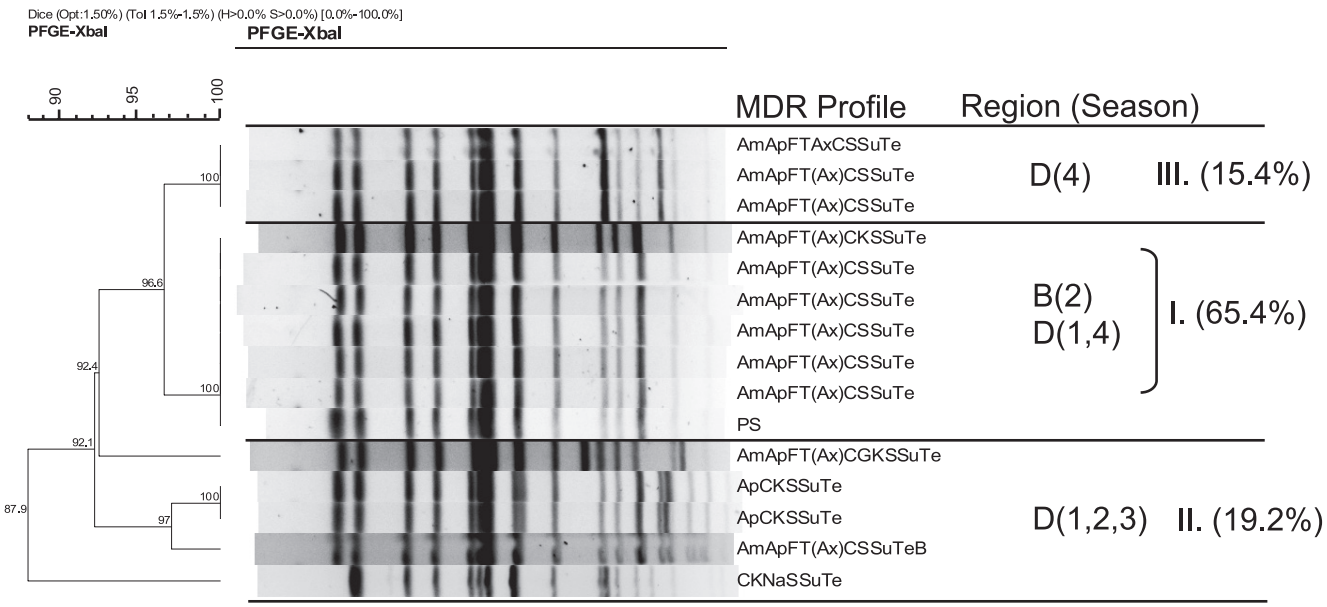

FIG. 6. XbaI PFGE-based dendrogram and MDR profiles of representative *Salmonella* Dublin isolates. Cluster analysis of banding patterns was performed using the Dice similarity coefficient and the unweighted-pair group method. Regions (B and D) and seasons (1, summer; 2, fall; 3, winter; 4, spring) where isolates were observed are indicated, in addition to the percentage of *Salmonella* Dublin isolates found in that cluster. Antimicrobial abbreviations: Am, amoxicillin-clavulanic acid; Ap, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; G, gentamicin; K, kanamycin; Sxt, sulfamethoxazole-trimethoprim; Cp, ciprofloxacin; N, nalidixic acid; and Ai, amikacin.

than that observed in plants in region A or B. And throughout the course of the study, MDR *Salmonella* prevalence values for region C were significantly lower ($P = 0.0008$) than those observed for region D (Table 2). Given that plants that slaughter cull cows and bulls typically obtain animals from a broad geographic area, our observation here of regional differences in MDR *Salmonella* prevalence is puzzling. It is possible that these data reflect the impact of plant lairage environments on cattle hide hygiene at harvest. However, these environments are constantly being seeded with new microorganisms by animals that often come from many parts of the country. Undoubtedly, the factors that influence bacterial competition and persistence in lairage environments (or in upstream environments such as auction markets or buying stations) represent important areas of further study, so that the impact of these factors on food safety can be assessed. However, it is also important to emphasize the limited nature of the study described herein, given that only one or two plants per region were sampled, for a total of nine sample days per region. Accordingly, the results presented here are by no means comprehensive. Rather, these data represent "snapshots" of what can be observed for MDR *Salmonella* prevalence in diverse locations overtime, which provide baseline information for further investigations into these complex systems.

In all, 16,218 *Salmonella* cattle hide and carcass isolates were collected and screened for antimicrobial resistance. The most commonly observed MDR *Salmonella* serotypes were Newport, Typhimurium, and Uganda, which collectively made up 80.6% of all MDR *Salmonella* strains characterized (Fig. 1). MDR *Salmonella* Newport resistance phenotypes were dominated by the MDR-AmpC phenotype (Fig. 3), while MDR *Salmonella* Typhimurium exhibited four basic resistance phenotypes (Fig. 4). These included the ACSSuTe phenotype that is typically associated with DT104, an expanded version of this phenotype that included resistance to aminoglycosides, the AKSSuTe phenotype that is frequently associated with DT193 or 208 (40, 61), and an expanded version of this phenotype including cephalosporin resistance. The isolates in the last group demonstrated XbaI PFGE profiles similar to those of the recently described MDR *Salmonella* Typhimurium clade WA-TYP035/187, reported by Adhikari et al., who also found these *Salmonella* Typhimurium strains to exhibit cephalosporin resistance (1), a phenotype that is generally uncommon in MDR *Salmonella* Typhimurium (52). The third most frequently isolated MDR *Salmonella* serotype was Uganda, and these isolates were found to demonstrate resistance to 12 of the 15 antimicrobials screened. All MDR *Salmonella* Uganda strains characterized were resistant to the cephalosporins ceftiofur and cefoxitin and showed either decreased sensitivity or resistance to ceftriaxone (Fig. 1).

In the past decade, MDR *Salmonella* strains have received increased attention from the medical community, especially those resistant to the extended-spectrum cephalosporin ceftriaxone, the drug of choice for the treatment of invasive salmonellosis in children (42). The emergence of cephalosporin resistance in *Salmonella* has been attributed to the spread of a large resistance plasmid containing the bla_{CMY2} gene (18, 38, 52, 59, 76). It has been suggested that extensive therapeutic use of the veterinary cephalosporin ceftiofur has been a major driving force for the dissemination of this resistance (20, 32), and there is concern that ceftiofur-resistant *Salmonella* strains may develop cross-resistance to ceftriaxone, because of the structural similarity of these two drugs. In this study, we found that 83.7% of the MDR *Salmonella* strains characterized were resistant to ceftiofur. Ceftriaxone resistance was observed less frequently, with only 12.1% of the isolates examined showing resistance to this antimicrobial; however, decreased susceptibility to ceftriaxone was detected in 66.9% of the isolates. Overall, the percentage of the total number of *Salmonella* strains characterized in this study found to be resistant to ceftriaxone was low (~5%, or 819 of 16,218 isolates). Nevertheless, veterinarians and cattle producers should take appropriate measures to minimize the spread of resistant pathogens when treating cattle with ceftiofur (28), as the selective pressure of antimicrobial exposure will cause subpopulations of any cephalosporin-resistant bacteria present to surge in number, possibly leading to an increase in the attempts to transfer these resistance determinants among bacterial populations.

Repetitive sampling at cattle slaughter establishments over the course of 10 months provided a unique opportunity to observe the diversity of MDR *Salmonella* strains present in these settings over time. Characterization of the serotypes, MDR phenotypes, and XbaI PFGE profiles of the *Salmonella* strains isolated revealed the existence of biotypes that appeared to be persistent and widely disseminated (observed in multiple seasons and from plants in multiple regions of the United States, as seen for *Salmonella* Newport and Typhimurium clusters I in Fig. 3 and 4, respectively) or endemic (observed in limited seasons or regions of the United States, as seen for *Salmonella* Newport cluster II [Fig. 3] and *Salmonella* Typhimurium clusters II to V [Fig. 4]). Hoelzer et al. recently reported a similar observation on the persistence and regional distribution of certain subtypes of *Salmonella* Newport and Typhimurium strains isolated from cattle and humans in two geographic regions of the United States (46). *Salmonella* Typhimurium and Newport are noted as host "generalists," meaning they are able to infect and cause disease in a wide range of host species (although *Salmonella* Typhimurium is also the mouse host-adapted serovar, causing murine typhoid fever). Accordingly, these serovars may be better able to spread or disseminate from one environment to the next, either by wild-animal movements or contaminated feed sources or by manure or agricultural waste runoff. Numerous studies have documented the ability of *Salmonella* Newport and Typhimurium strains to survive in manure and terrestrial environments for extended periods (upwards of 9 months) (10, 13, 25, 77). Of course, genome-wide analyses would be needed to establish the relatedness of isolates in the clusters identified here, as a single PFGE profile is not a definitive indicator of lineage or relatedness (33, 46). However, the observation of potentially epidemic and endemic biotypes in this study further demonstrates the need, mentioned above, for research into the ability of pathogens to persist in epidemiologically complex, agricultural settings.

A final observation concerns comparisons of the *Salmonella* serotypes isolated from cattle hides versus those isolated from postintervention carcasses in this study. Preliminary typing data on all *Salmonella* strains collected in this study indicate that the dominant serotypes found entering processing environments on cattle hides were Montevideo, Anatum, and

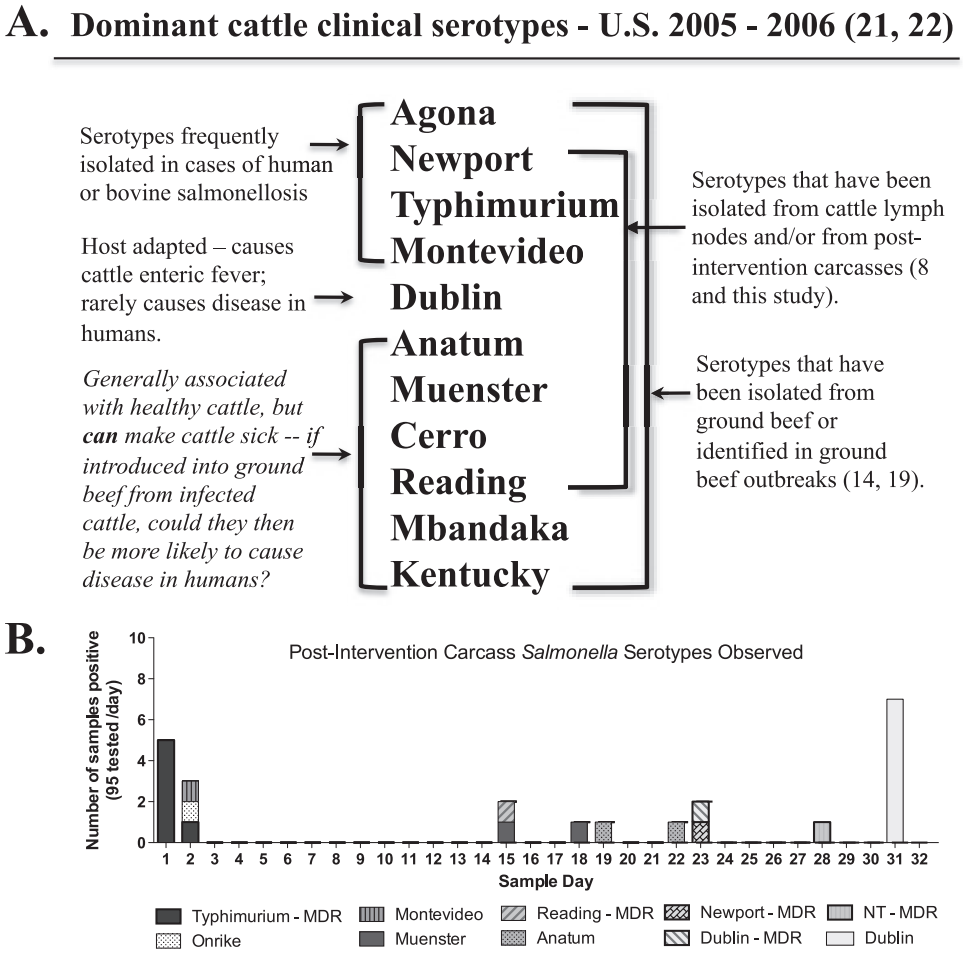

FIG. 7. (A) Comparison of the serotypes most frequently isolated from clinically infected cattle with those noted for frequently causing disease in humans and those isolated from cattle lymph nodes, ground beef, and postintervention carcasses. (B) Graph depicting the distribution of *Salmonella* serotypes isolated from postintervention carcasses by sample day.

Muenster. These data are in keeping with the observations of Kunze et al., who found *Salmonella* Anatum and Montevideo to be the predominant serotypes on cattle hides at harvest (51). These serotypes are noted as being frequently isolated from healthy cattle (17, 74), and yet they were not the predominant serotypes found on postintervention carcasses here. As seen in Fig. 7B, postintervention carcass contamination was generally sporadic. However, on two separate occasions, clusters of postintervention carcasses contaminated with *Salmonella* were observed. The *Salmonella* strains found contaminating these carcasses were those of MDR *Salmonella* Typhimurium in one event and pansusceptible *Salmonella* Dublin in the other, both serotypes known for causing clinical illness in cattle.

This observation prompted a comparison of the *Salmonella* serotypes reported in a number of studies, including serotypes found in ground beef (14, 75, 79), those isolated from cattle lymph nodes (8), and those found on final carcasses in this study, along with serotypes noted for being isolated from clinically infected cattle (21, 22). The last group was identified from the *Salmonella* Surveillance Annual Summary reports for 2005 and 2006, which summarize the *Salmonella* strains isolated and serotyped from clinical cases of animal disease that were reported to the Centers for Disease Control and Prevention (CDC) and the National Veterinary Services Laboratory (NVSL) in those time periods. The CDC cautions that samples from nonhuman sources that are tested for *Salmonella* are obtained in a variety of ways and that sampling is neither complete nor random and undoubtedly has biases. Nevertheless, the same 11 serotypes were identified as causing more than 70% of reported bovine salmonellosis in 2005 (n = 2,674) and 2006 (n = 3,770) (82.6% and 72.9%, respectively). In comparing these 11 serotypes with those found in ground beef, in cattle lymph nodes, and on final carcasses, we found considerable overlap in the serotype distribution of these data sets (Fig. 7A).

Salmonella strains entering beef processing environments on the hides or in the feces of cattle, weather they are MDR or not, are undoubtedly present in a variety of metabolic states. The metabolic state of an organism, in combination with its genetic makeup (in terms of the stress response and virulence genes present), strongly influences the ability of that organism to respond to environmental stressors. One of the ways that *Salmonella* enters beef processing environments is possibly via

cattle that are *Salmonella* carriers. It is well known that *Salmonella* infection can lead to the development of a carrier state (47, 69). After primary challenge, cattle may become passive carriers (immune animals demonstrating no active pathology that excrete *Salmonella* acquired from contaminated environmental sources), active carriers (animals excreting high levels of *Salmonella*, often in the absence of clinical signs), or latent carriers (asymptomatic animals with persistent *Salmonella* infection present in tissues) (69). *Salmonella* carriers that enter beef processing environments are likely shedding *Salmonella* strains that have survived the host environment. These *Salmonella* strains could, consequently, be adapted to acidic pH or exposure to thermal extremes (16) and as a result would be better equipped to survive carcass processing interventions. This could account for the preponderance of serotypes noted for infecting cattle on postintervention carcasses and in ground beef. The data presented here highlight the need for a better understanding of the biology of *Salmonella* carrier status in cattle and of the metabolic state(s) of the *Salmonella* being shed by carriers. Research in these areas will provide vital information for future efforts aimed at controlling *Salmonella* in cattle production and processing environments.

ACKNOWLEDGMENTS

This project was funded in part by The Beef Checkoff.

We thank the plant personnel for their kind assistance and cooperation. We also thank Michael Guerini for his contribution to this project, Julie Dyer, Frank Reno, Bruce Jasch, Greg Smith, Kim Kucera, Jade Franklin, Casey Trambly, Sara Schumacher, and Emily Griess for their technical support, and Joan Rosch for administrative assistance.

The use of product names is necessary for factual reporting on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA does not imply approval of the product to the exclusion of others that may also be suitable.

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