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Characterization of Antimicrobial-Resistant Phenotypes and Genotypes among *Salmonella enterica* Recovered from Pigs on Farms, from Transport Trucks, and from Pigs after Slaughter

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ABSTRACT

The main objectives of this study were to determine antimicrobial resistance patterns among *Salmonella* serotypes and to evaluate the role of transport trucks in dissemination of antimicrobial-resistant strains of *Salmonella*. *Salmonella* from groups of nursery and finishing pigs on farms, from trucks, and from pigs after slaughter were compared using serotyping, patterns of antimicrobial resistance, and pulsed-field gel electrophoresis patterns. The five farms included in the study yielded 858 isolates representing 27 *Salmonella* serovars. The most common resistance observed (80% of all isolates) was to tetracycline; resistance to ampicillin (42%), chloramphenicol (31%), amoxicillin/clavulanic acid (30%), and piperacillin (31%) also were common. We found a correlation between serovar and antimicrobial resistance. High correlation was found between *Salmonella* Typhimurium var. Copenhagen and chloramphenicol resistance (Spearman rank correlation, $\rho = 0.7$). Multidrug resistance was observed primarily in *Salmonella* Typhimurium var. Copenhagen (94%) and *Salmonella* Typhimurium (93%) and was much less common in the other common serovars, including *Salmonella* Derby (7%) and *Salmonella* Heidelberg (8%). Of the 225 isolates exhibiting the most common pentaresistance pattern in this study, amoxicillin/clavulanic acid–ampicillin–chloramphenicol–piperacillin–tetracycline, 220 (98%) were *Salmonella* Typhimurium var. Copenhagen, and 86% of the isolates of this serovar had this pattern. Isolates from the trucks were similar, based on pulsed-field gel electrophoresis patterns, to those from the cecum and mesenteric lymph nodes of pigs on two of the farms, suggesting the probable infection of pigs during transport. Class I integrons were also common among various serovars.

Salmonella is an important and ubiquitous foodborne pathogen *(5).* Rapid emergence and dissemination of antimicrobial-resistant organisms has become a public health concern of global proportions *(20, 30).* Recommendations by the World Organization for Animal Health *(42)* and the World Health Organization *(41)* have reaffirmed the potential for transfer of antimicrobial-resistant organisms from food animals to humans and the need for standardized surveillance, research on identification of risk factors, and prudent use of antimicrobials. Foodborne pathogens, including *Salmonella,* are frequently a focus of such discussions because foodborne infection is an obvious route for transmission of resistant organisms.

Antimicrobial use is widespread in swine production in North America *(7, 10–12).* According to the National Animal Health Monitoring System report, >88% of swine farms in the United States use antimicrobials at grower-finisher phases. The majority of antimicrobials are used at subtherapeutic levels for purposes of growth promotion. Tylosin, chlortetracycline, and bacitracin are the three most common antimicrobials in swine diets. Procaine penicillin G and tylosin are also the two most common antimicrobials used at therapeutic levels parenterally. Although not as common, ceftiofur, a third-generation cephalosporin, is also used at therapeutic levels *(26).* Recently, baseline data on antimicrobial resistance of indicator organisms and zoonotic organisms and findings on some animal pathogens were published as part of a comprehensive surveillance system in Denmark *(1, 2)* and the United States *(28).* Resistance patterns differed between indicator organisms and zoonotic pathogens. These data suggest that epidemiologic studies of resistance in foodborne pathogens should be conducted on the pathogens themselves rather than on indicator organisms, such as *Escherichia coli.* The National Antimicrobial Resistance Monitoring System *(27, 28)* in the United States has similarly reported that the frequency of antimicrobial resistance among *Salmonella* isolates from swine is higher than that among isolates from chickens and beef cattle for 14 and 8 of the 17 antimicrobials tested, respectively.

The objectives of this study were to determine antimicrobial resistance patterns among *Salmonella* serotypes and to evaluate the role of transport trucks in dissemination of antimicrobial-resistant strains of *Salmonella* by comparing serovars, patterns of antimicrobial resistance, and pulsed-field gel electrophoresis (PFGE) patterns of *Salmo-*

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nella isolates from groups of nursery and finishing pigs on farms and after slaughter.

MATERIALS AND METHODS

Salmonella isolates were available from two independent studies conducted by in North Carolina from 1998 to 2000. Study 1 (farms 1–4) was originally designed for the purposes presented here. Study 2, however, had independent objectives, as described previously *(25).* Because the two studies had sample design features in common, with groups of pigs sampled on the farm and then followed to slaughter, we included the data from study 2 as farm 5 for the present study. On all farms, finishing pigs were reared in confinement with similar management and feed conditions.

Truck swab collection. Trucks that transported the groups of pigs studied were swabbed using sterile sponges immersed in buffer peptone water (Difco Laboratories, Becton Dickinson, Sparks, Md.). These trucks were provided by the slaughter plant, and swabs were collected at two stages: at the remote truck wash station (not on the farm) after the trucks had been cleaned (preload sampling) and at the processing plant immediately after the transported pigs had been moved into the lairage area (postload sampling). Each sampling involved swabbing the trucks at four of the corners and in the middle of the lower deck. Personnel collecting the swabs wore sterile coveralls, and aseptic measures were taken to prevent external contamination.

Bacterial culture and selection of isolates. We used conventional techniques for culture and isolation of *Salmonella* as described previously *(8, 15).* Separate protocols for culture of fecal samples for *Salmonella* were used in study 1 (farms 1–4) *(15)* and study 2 (farm 5) as described previously *(8).* However, culture methods were uniform within studies, and the two methods have been shown to yield similar results *(9).* Fecal samples (10 g) were preenriched using Hajna broth (study 1) or buffered peptone water (Becton Dickinson, Sparks, Md.) (study 2) and then incubated at 37°C for 24 h. A 100-μl suspension from each sample was transferred to Rappaport Vassillidas medium (Difco) at 1:100 dilutions and incubated at 42°C for 24 h. Samples were then plated on Bacto XLT-4 agar base (Difco) and incubated at 37°C for 24 h. Single colonies were then tested for the appropriate biochemical reactions on triple sugar iron agar and urea agar (Difco). Serotyping was done at the National Veterinary Services Laboratories (Ames, Iowa).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on all isolates from farms 1–4. For farm 5, susceptibility testing was conducted on 162 isolates from on-farm fecal samples and 165 isolates from cecal cultures obtained after slaughter. When more than five isolates of a given serovar were recovered on a single day (study 2), five of the isolates were randomly selected for testing. Antimicrobial susceptibility was tested using the Vitek Jr. (bioMérieux, Hazelwood, Mo.) semiautomated antimicrobial panel system for 11 antimicrobials as per the manufacturer's directions. The antimicrobials and breakpoints for resistance were amikacin (Ak), 64 μg/ml; amoxicillin/clavulanic acid (Ax), 32 μg/ml; ampicillin (Am), 32 μg/ml; cefotaxime (Cf), 32 μg/ml; cephalothin (Ce), 32 μg/ml; chloramphenicol (Cm), 32 μg/ml; ciprofloxacin (Cip), 4 μg/ml; gentamicin (Gm), 16 μg/ml; piperacillin (Pi), 128 μg/ml; tetracycline (Te), 16 μg/ml; and trimethoprim-sulfamethoxazole (Tr/Su), 4/76 μg/ml. Bacterial resistance was determined by using NCCLS standards for gram-negative enteric organisms, and the following quality control strains were used: *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 *(29).*

PFGE fingerprinting. PFGE fingerprinting was done as recommended by the Pulsenet, Centers for Disease Control and Prevention *(16)* as described previously. Overnight culture cells (200 μl) were lysed and digested with a restriction enzyme, *Xba*I. The digested agarose-embedded DNA was then separated using a CHEF-DRIII (Bio-Rad, Hercules, Calif.) apparatus. The gel was stained in ethidium bromide (1 μg/ml), and a Gel Doc 2000 (Bio-Rad) was used to capture fingerprint images.

Identification of class I integrons. Variable region of class I integrons was amplified using the following PCR primers: 59-CS (59-GGCATCCAAGCACAAGC-39) and 39-CS (59-AAGCAGACTTGACCTGAT-39). The lowest expected size of the variable region if no resistance gene cassette is inserted in the integron was 153 base pairs. Amplification reactions were carried out with 1 μl of purified DNA (DNAeasy tissue kit, Qiagen, Valencia, Calif.), 300 μM deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 50 pmol of primers, and 0.5 U of Gold *Taq* polymerase (Perkin-Elmer, Foster City, Calif.). Distilled water was added to bring the final volume to 20 μl. The PCR cycle included initial denaturation for 5 min at 95°C and 30 cycles of denaturation for 1 min at 95°C, primer annealing for 1 min at 54°C, and extension for 1 min at 72°C.

Analysis. The association between the proportion of serovars at each sampling stage and frequency of antimicrobial resistance was tested using Spearman rank correlation *(21).* Analysis of PFGE data was performed using Bionumerics software (Applied Maths, Keistraat, Belgium), and dendrograms were drawn using the unweighted pair group method of arithmetic averages.

RESULTS

Antimicrobial resistance of *Salmonella* isolates. To investigate resistance to antimicrobial agents in strains of *Salmonella,* we collected fecal samples from pigs on farms, swabs from trucks transporting pigs, and cecal and mesenteric lymph node samples from the transported pigs after slaughter. The five farms yielded 858 isolates from 27 *Salmonella* serovars. The most common resistance observed (80% of all isolates) was to tetracycline; resistance to ampicillin (42%), chloramphenicol (31%), amoxicillin/clavulanic acid (30%), and piperacillin (31%) also was common (Table 1). None of the isolates were resistant to ciprofloxacin, amikacin, or a third-generation cephalosporin (cefotaxime). Resistance to tetracycline and β-lactams was common regardless of the stage of sampling; resistant isolates were obtained from farms, trucks, and slaughter plants. However, frequency of resistance was correlated with the proportion of the specific serovar. A moderate correlation was found between the proportion of *Salmonella* Typhimurium (var. Copenhagen) and the frequency of tetracycline resistance ($\rho = 0.3$). However, a high correlation ($\rho = 0.7$) was found between *Salmonella* Typhimurium (var. Copenhagen) and chloramphenicol resistance.

Although the number of isolates collected from transport swabs was low, a higher proportion of trimethoprim-sulfamethoxazole resistance (11%) was observed. Resistance to this antimicrobial was uncommon at the other stages; <1% of isolates recovered from samples collected 48 h before slaughter and at slaughter and 3% of those collected

TABLE 1. *Number and percentage of antimicrobial-resistant* Salmonella *isolates from pigs and trucks from five farms in North Carolina*

Sampling stage[a]	Serovar	No. tested	No. (%) of isolates resistant to[b]: Te	Am	Cm	Ax	Pi	Gm	Ce	Tr/Su
F_{1-3}	Typhimurium (var. Copenhagen)	39	32 (82)	39 (100)	31 (80)	39 (100)	34 (87)	0	0	0
	Derby	92	84 (91)	15 (16)	14 (15)	1 (1)	3 (3)	15 (16)	0	6 (7)
	Typhimurium	61	58 (95)	57 (93)	1 (2)	5 (8)	13 (21)	0	1 (2)	0
	Heidelberg	15	13 (87)	0	0	0	0	0	0	2 (13)
	Others (10 serovars)	71	7 (10)	1 (1)	3 (4)	1 (1)	1 (1)	0	3 (4)	0
	Subtotal	278	194 (70)	112 (40)	49 (18)	46 (16)	50 (18)	15 (5)	4 (1)	8 (3)
F_4	Typhimurium (var. Copenhagen)	35	35 (100)	35 (100)	33 (94)	35 (10)	35 (100)	0	0	0
	Derby	27	27 (100)	3 (11)	1 (4)	1 (4)	0	1 (4)	1 (4)	0
	Typhimurium	19	18 (95)	19 (100)	3 (16)	3 (16)	4 (21)	0	1 (5)	0
	Heidelberg	4	4 (100)	0	0	0	0	0	0	0
	Others (two serovars)	4	0	0	0	0	0	0	0	0
	Subtotal	89	84 (94)	57 (64)	37 (42)	39 (44)	39 (44)	1 (1)	2 (2)	0
S_{LND}	Typhimurium (var. Copenhagen)	74	74 (100)	74 (100)	73 (99)	72 (97)	72 (97)	1 (1)	0	0
	Derby	17	14 (82)	0	1 (6)	0	0	0	0	0
	Typhimurium	10	9 (90)	10 (100)	1 (10)	1 (10)	5 (50)	1 (10)	0	0
	Heidelberg	9	9 (100)	0	0	0	0	0	0	0
	Others (six serovars)	19	4 (21)	0	0	0	0	0	0	1 (5)
	Subtotal	129	110 (85)	84 (65)	75 (58)	73 (57)	77 (59)	2 (2)	0	1 (<1)
S_{cecal}	Typhimurium (var. Copenhagen)	103	103 (100)	88 (85)	86 (83)	88 (85)	87 (84)	0	0	0
	Derby	118	90 (76)	2 (2)	7 (6)	1 (1)	2 (2)	1 (1)	0	0
	Typhimurium	15	15 (100)	14 (93)	2 (13)	3 (20)	7 (47)	0	0	0
	Heidelberg	37	36 (97)	2 (5)	3 (8)	2 (5)	2 (5)	0	0	2 (5)
	Others (18 serovars)	80	39 (49)	1 (1)	1 (1)	1 (1)	0	0	0	2 (2)
	Subtotal	353	283 (80)	99 (28)	99 (28)	95 (27)	98 (28)	1 (<1)	0	4 (1)
Transport	Typhimurium (var. Copenhagen)	6	6 (100)	5 (83)	5 (83)	5 (83)	4 (67)	0	0	1 (17)
	Heidelberg	1	1 (100)	0	0	0	0	0	0	0
	Others (two serovars)	2	0	0	0	0	0	0	0	0
	Subtotal	9	7 (78)	5 (56)	5 (56)	5 (56)	4 (44)	0	0	1 (11)
	Total	858	678 (80)	365 (42)	265 (31)	258 (30)	268 (31)	19 (2)	6 (<1)	14 (2)

[a] F_{1-3}, farm samples from pigs at approximately 4, 15, and 22 weeks of age, respectively; F_4, farm samples taken from market-age pigs within 48 h of slaughter; S_{LND}, mesenteric lymph node samples from slaughter pigs; S_{cecal}, cecal tissue and content samples from slaughter pigs.

[b] Te, tetracycline; Am, ampicillin; Cm, chloramphenicol; Ax, amoxicillin/clavulanic acid; Pi, piperacillin, Gm, gentamicin; Ce, cephalothin; Tr/Su, trimethoprim-sulfamethoxazole. No resistance to amikacin, cefotaxime, and ciprofloxacin was detected.

TABLE 2. *Frequency distribution of* Salmonella *isolates (*n *= 858) by patterns of resistance to 11 antimicrobials*

Resistance pattern[a]	No. (%) of isolates	No. (%) of isolates by *Salmonella* serovar				
		Typhimurium var. Copenhagen (*n* = 257)	Derby (*n* = 254)	Typhimurium (*n* = 105)	Heidelberg (*n* = 66)	Others (23 serovars) (*n* = 176)
None	159 (19)	0	32 (13)	3 (3)	1 (2)	123 (70)
Te	318 (37)	15 (6)	196 (77)	2 (2)	60 (91)	45 (26)
Am	2 (<1)	0	0	2 (2)	0	0
Ce	2 (<1)	0	0	0	0	2 (1)
AmTe	63 (7)	1 (<1)	1 (<1)	61 (58)	0	0
TeTr/Su	6 (<1)	1 (<1)	0	0	2 (3)	3 (2)
TeCm	7 (<1)	0	6 (2)	0	1 (2)	0
CeCm	2 (<1)	0	0	0	0	2 (1)
AmGm	1 (<1)	0	1 (<1)	0	0	0
AmTePi	21 (2)	0	0	21 (20)	0	0
AmAxTe	6 (<1)	1 (<1)	0	5 (5)	0	0
AxAmPi	7 (<1)	7 (3)	0	0	0	0
AxCmTe	2 (<1)	0	0	1 (1)	0	1 (<1)
AmGmTe	2 (<1)	0	1 (<1)	1 (1)	0	0
AxAmCmTe	8 (<1)	7 (3)	0	1 (1)	0	0
AxAmPiTe	6 (<1)	4 (2)	0	2 (2)	0	0
AxAmCeTe	1 (<1)	0	1 (<1)	0	0	0
AmCmGmTe	11 (1)	0	11 (4)	0	0	0
AmCmPiTe	2 (<1)	0	0	2 (2)	0	0
AmCmGmPiTe	3 (<1)	0	3 (1)	0	0	0
AxAmCmPiTe	225 (26)	220 (86)	1 (<1)	2 (2)	2 (3)	0
AxAmCePiTe	1 (<1)	0	0	1 (1)	0	0
AxAmCmGmPiTe	2 (<1)	1 (<1)	1 (<1)	0	0	0
AxAmCeCmPiTe	1 (<1)	0	0	1 (1)	0	0

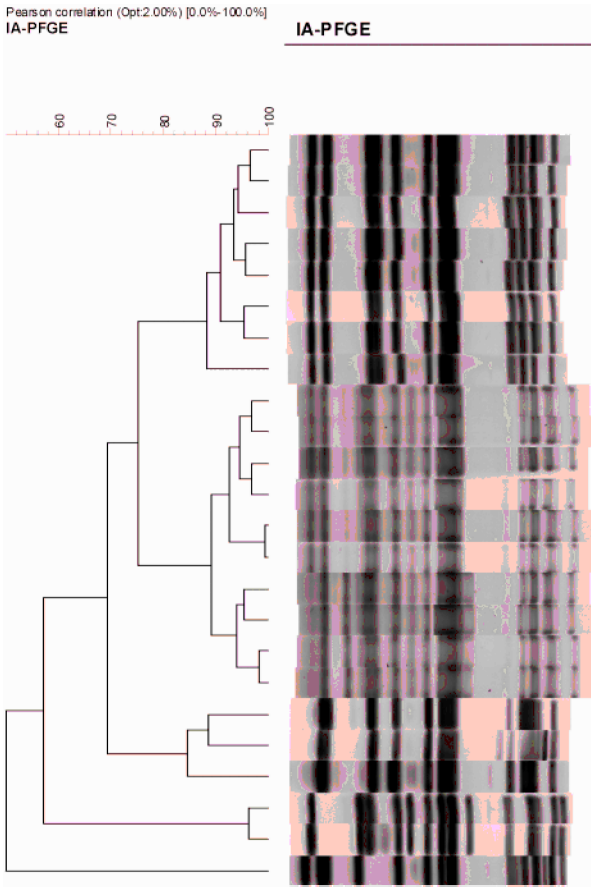
[a] Te, tetracycline; Am, ampicillin; Cm, chloramphenicol; Ax, amoxicillin/clavulanic acid; Pi, piperacillin; Gm, gentamicin; Ce, cephalothin; Tr/Su, trimethoprim-sulfamethoxazole.

in early stages on farms were resistant to this antimicrobial combination. Resistance to gentamicin was also uncommon; resistance often was associated with *Salmonella* Derby (15 of total 19 isolates) and was found in isolates from samples collected at early stages on farms (Table 1).

Serovars and resistance patterns. Resistance to multiple antimicrobials was also frequently observed; 44% of the isolates were resistant to more than one antimicrobial (Table 2). Of the 858 isolates tested, 159 (19%) were susceptible to all 11 antimicrobials tested. Among isolates resistant to one or more antimicrobials, the most common resistance pattern (37% of isolates) was resistance to tetracycline alone. Combinations of resistance typically involved tetracycline and often involved ampicillin. There was a striking association between resistance pattern and serovar. For example, of the 225 isolates exhibiting the AxAmCmPiTe resistance phenotype, 220 (98%) were *Salmonella* Typhimurium var. Copenhagen, and 86% of isolates of this serovar had this pattern (Table 2). Similar examples of resistance patterns associated with individual serovars were found for three other prevalent serovars. For *Salmonella* Derby and *Salmonella* Heidelberg, isolates resistant to tetracycline alone represented 77 and 91% of all isolates of these respective serovars. For *Salmonella* Typhimurium, 58% of isolates were resistant to ampicillin and tetracycline, and an additional 20% were resistant to those two antimicrobials and to piperacillin (Table 2).

The four most common serovars (*Salmonella* Typhimurium var. Copenhagen, *Salmonella* Derby, *Salmonella* Typhimurium, and *Salmonella* Heidelberg) accounted for 80% of all isolates, and 54% of these isolates were resistant to more than one antimicrobial. However, multidrug resistance was observed primarily in *Salmonella* Typhimurium var. Copenhagen (94%) and *Salmonella* Typhimurium (93%) and was much less common in *Salmonella* Derby (7%) and *Salmonella* Heidelberg (8%). Among the remaining 23 serovars, 69% of isolates were susceptible to all antimicrobials tested, an additional 26% were resistant only to tetracycline, and 6 isolates (3%) exhibited resistance to two or more antimicrobials.

PFGE patterns of isolates from truck and slaughter plant samples. PFGE was used to determine whether infection during transport could contribute to changes in the composition of *Salmonella* Typhimurium and Typhimurium var. Copenhagen PFGE patterns present in samples collected at slaughter. Comparison of isolates with similar PFGE patterns of this serovar might suggest contamination during transport and association with isolates collected during slaughter. Isolates that were collected from trucks before and after loading pigs were compared with those from



Isolate Id	Serovar[a]	R-Pattern	Farm #	Source	Sample type
IR38	Copenhagen	AmCmTeAx	1	Slaughter	Cecum
IR9	Copenhagen	AmCmPiTeAx	1	Truck Swab	Preload
IS5	Copenhagen	AmCmPiTeAx	2	Slaughter	Cecum
IS16	Copenhagen	AmCmPiTeAx	2	Slaughter	Lymph node
IS21	Copenhagen	AmCmPiTeAx	2	Slaughter	Cecum
IS1	Copenhagen	AmCmPiTeAx	2	Slaughter	Cecum
IS14	Copenhagen	AmCmPiTeAx	2	Truck Swab	Preload
IR46	Copenhagen	AmCmTeAx	1	Truck Swab	Preload
IAD13	Typhimurium	AmTe	3	Farm (Nursery)	Fecal
IAD3	Copenhagen	AmCmTeAx	3	Farm (Nursery)	Fecal
IAD1	Copenhagen	AmCmPiTeAx	3	Farm (Nursery)	Fecal
IAH24	Typhimurium	AmTe	3	Farm(15 weeks)	Fecal
IAI2	Copenhagen	AmCmPiTeAx	4	Farm(22 weeks)	Fecal
IAH4	Copenhagen	AmCmPiTeAx	3	Farm(15 weeks)	Fecal
IAE2	Copenhagen	AmCmTeAx	4	Farm(15 weeks)	Fecal
IAE6	Copenhagen	AmCmTeAx	4	Farm(15 weeks)	Fecal
IAI11	Copenhagen	AmCmPiTeAx	4	Farm(22 weeks)	Fecal
IAI10	Copenhagen	AmCmPiTeAx	4	Farm(22 weeks)	Fecal
IAP18	Typhimurium	AmTe	3	Farm (Preslaughter)	Fecal
IAP11	Typhimurium	AmAxTe	3	Farm (Preslaughter)	Fecal
IAP17	Typhimurium	AmPiTe	3	Farm (Preslaughter)	Fecal
IAP15	Typhimurium	AmCmTe	3	Farm (Preslaughter)	Fecal
IAP23	Typhimurium	AmCmCfPiTeAx	3	Farm (Preslaughter)	Fecal
IR47	Copenhagen	TeTr/Su	1	Truck Swab	Postload

FIGURE 1. *PFGE fingerprint and dendrogram of 14 isolates collected from three farms (groups of pigs), showing similarity between transport and slaughter isolates.* [a]*Copenhagen refers to serovar* Salmonella *Typhimurium var. Copenhagen.*

the lymph nodes and cecum from the transported pigs. This analysis was performed on isolates from pigs derived from all four groups, although the main interest was in farms 1 and 2 because the corresponding trucks tested positive for *Salmonella* before the pigs were loaded.

Isolates from fecal samples collected on the farm had fingerprint types that were distinctly different from those isolates from truck and slaughter samples (Fig. 1). Isolates from the trucks were genotypically identical to those from the cecum and mesenteric lymph nodes of the group of pigs at two of the farms. For example, isolate IR9 from a truck and isolate IR38 from the cecum of a farm 1 pig eventually transported on that truck were identical in their resistance patterns and had closely related PFGE patterns (Fig. 1). This finding suggests that isolates obtained from trucks prior to the transport of the tested groups of pigs did infect these animals during transport. In addition, one isolate (IR47) that was cultured from the truck had a fingerprint different from that of isolates from samples collected at the slaughter plant, with <60% band identity, and had the TeTr/Su resistance pattern (Fig. 1). This resistance pattern had not been detected in slaughtered pigs or from pigs on farms. The PFGE pattern was also distinct from others commonly found in other swine isolates, suggesting that *Salmonella* can survive the cleaning of trucks, allowing the infection of subsequent groups of pigs. From farm 2, one clonal isolate was obtained from truck swabs and from the cecum and the mesenteric lymph node of slaughtered pigs (isolates IS1, IS5, IS14, IS16, and IS21). For farms 1 and 2, *Salmonella* Typhimurium had not been found on the farms during repeated sampling of pigs (at 4, 15, and 22 weeks of age) and at 48 h before slaughter (preslaughter). Figure 1 also shows 15 additional isolates from farms 3 and 4, from which *Salmonella* Typhimurium and *Salmonella* Typhimurium var. Copenhagen were frequently isolated, indicating that the *Salmonella* Typhimurium isolates obtained from pigs on farms in this study are different from those obtained at slaughter.

Class I integrons. In this study, analysis of integrons was performed on 15 isolates of six *Salmonella* serovars, including Derby (AmCmGmTe), Worthington (AxAmCePiTe), Muenchen (AxAmCmTe), Muenster (AxAmCeGmPiTe and GmTe), Bere (AmTe), and Typhimurium var. Copenhagen (Te). All the serovars had at least one class I integron (Fig. 2). Three integrons per isolate were detected among isolates of serovar Worthington, ranging between 200 bp and 1.6 kb. The hexaresistant Muenster isolates carried the largest integron, about 2 kb in size.

DISCUSSION

The primary purpose of this study was to determine the antimicrobial resistance of *Salmonella* serovars isolated from pigs both on farms and at slaughter and to utilize

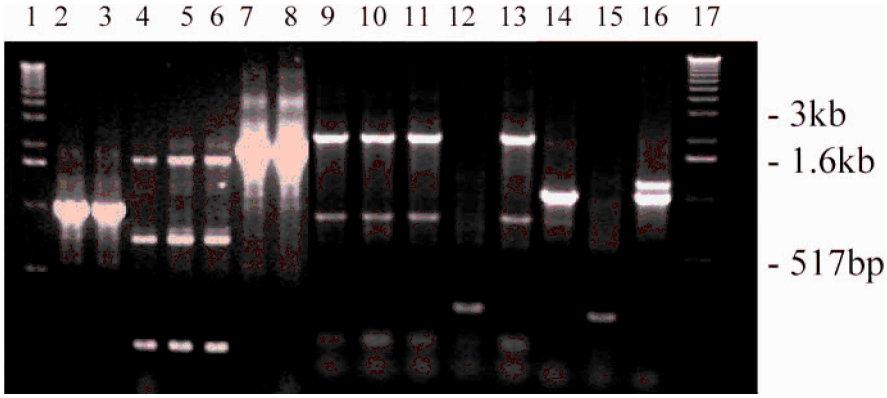


FIGURE 2. *PCR amplification of the variable region of a class I integron among six* Salmonella *serovars. The lanes and representative isolates are as follows. Lanes 1 and 17, molecular markers; lanes 2 and 3, serovar Derby; lanes 4 to 6, serovar Worthington; lanes 7 and 8, serovar Muenchen; lanes 9 to 11, 13, and 14, serovar Muenster; lane 12, serovar Copenhagen; lane 15, serovar Bere; lane 16, serovar Typhimurium.*

antimicrobial resistance patterns of predominant serovars as a basis for phenotypic classification. Consistent with other recent findings in the United States and other countries, resistance to tetracycline and β-lactams was common *(11, 12, 14, 24, 27, 28, 37)*; 80% of the total 858 isolates were resistant to tetracycline. None of the isolates were resistant to ciprofloxacin, a fluoroquinolone to which resistance has been reported among *Salmonella* isolates in Europe *(38)*. Recently, data from the National Antimicrobial Resistance Monitoring System in the United States also revealed the presence of fluoroquinolone-resistant *Salmonella* isolates in clinical human salmonellosis cases *(31, 34)*.

In the present study, no resistance to third-generation cephalosporins was detected. Resistance to this class of antimicrobial has increasingly been reported primarily among human isolates of various serovars *(13, 32, 39, 40)*. One notable serovar often associated with third-generation cephalosporin resistance is serovar Newport. In recent years, strains of this serovar have been found to carry multidrug resistance plasmids conferring resistance to third-generation cephalosporins *(3, 43)*. Disease outbreaks due to this serovar have often been associated with bovine *(33, 43)* or vegetable *(22)* products and are extremely rare in swine. Consistent with previous reports, only a single isolate of *Salmonella* Newport was detected in this study. Unlike many of the recently reported *Salmonella* Newport isolates, however, this isolate was susceptible to all of the 11 antimicrobials tested.

Antimicrobial resistance was common in both on-farm and after-slaughter samples. Slight differences in frequency of resistance to each antimicrobial were noticed among the sampling stages (Table 1). However, such differences probably are due to the serovar composition of isolates obtained from each stage. We found a correlation between serovars and resistance. Of particular interest is the high correlation ($\rho = 0.7$) of the most common serovar Typhimurium (var. Copenhagen) with chloramphenicol resistance. This resistance is due mainly to the high occurrence of phage type DT104, which often exhibits resistance to chloramphenicol and to four other antimicrobials *(38)*. Others *(6)* also have suggested the use of this phenotype as a primary screening approach for detecting phage type DT104.

Multidrug resistance was found in 94% of 362 isolates of *Salmonella* Typhimurium (including var. Copenhagen), 220 of which showed the pentaresistance pattern AxAmCmPiTe. Even though this serovar was very common (225 isolates of serovar Typhimurium var. Copenhagen among the study pigs), no overt clinical signs were noticed. Similarly, in a longitudinal study of one swine farm in Japan from 1972 to 1974 multiple resistance was found in 68% of *Salmonella* Typhimurium isolates compared with 7% of isolates of 4 other serovars *(23)*. More frequent occurrence of multidrug resistance in *Salmonella* Typhimurium compared with other serovars has also been observed in other recent studies in the United States *(4, 17, 18, 27)*. The authors of these reports commented that differences in resistance patterns among isolates of different origins (feces, lymph nodes, ceca) may be attributable to differences in serovar distribution.

The findings of the present study show that resistant *Salmonella* Typhimurium and Typhimurium (var. Copenhagen) isolates were widespread among the tested farms throughout the sampling periods. Such multidrug-resistant strains were also commonly found at slaughter. Multidrug resistance was frequently found among the serovars most often associated with human infections, particularly *Salmonella* Typhimurium. The common but nonhuman-associated serovars such as *Salmonella* Derby were predominantly susceptible to all antimicrobials.

In this study, PFGE genotyping was performed on 24 isolates (Fig. 1). This fingerprinting method was used to investigate the genetic similarity between isolates collected from trucks and those collected after slaughter; these isolates may be indistinguishable using phenotypic methods such as serotyping and antimicrobial resistance patterns. On two of the four farms, we found isolates from the cecum and lymph nodes of slaughtered pigs that were clones of isolates collected from trucks before loading the pigs. The trucks were supplied by the slaughter plant and cleaned at locations distant from the farms, and swabbing was also performed at locations remote from the farms. The genotypic and phenotypic similarity between isolates from trucks and those from slaughter samples is interesting because this serovar has not been detected on either of the farms. The clonality of these isolates may therefore be suggestive of contamination during transport. However, we acknowledge the limitations of microbiological assays, in that the strains may have been on the farm but had not been detected by our sampling techniques. Two studies have recently been published that involve the use of genotypic ap-

proaches to characterize sources of *Salmonella* contamination in slaughtered pigs *(19, 35, 36).* In one study *(19),* PFGE patterns of *Salmonella* Typhimurium were common to two plants tested and to on-farm sources. Although the authors did not state whether the isolates were of var. Copenhagen, the findings at the serovar level were consistent with those of the present study. No direct comparison with our study is possible with regard to the role of trucks, because the sampling in the other study did not involve truck swabs. Other studies *(35, 36)* involved sampling on the farm, on trucks, and after slaughter. These authors concluded that the farm and slaughter environment, particularly lairage, are important sources of *Salmonella* contamination in pig carcasses. Thus, our findings are consistent with those of others who found that pigs can become contaminated with *Salmonella* after leaving the farm, explaining the contamination found at slaughter.

Analysis of class I integrons using PCR of genomic regions that are variable among serovars with various resistance phenotypes revealed that these mobile DNA elements are common. Each of the five serovars tested carried at least one integron sequence in the genome. The resistance patterns of the non–multidrug-resistant isolates, such as those of serovars *Salmonella* Typhimurium var. Copenhagen (lane 12, Te) and *Salmonella* Bere (lane 15, AmTe), had the smallest integron variable region, suggesting that no resistance gene cassette is present in this integron; rather, the resistance genes may be located out of the integron variable region. In contrast, the hexaresistant *Salmonella* Muenster isolates carried the largest integron, about 2 kb, indicating that the integron may have carried multiple resistance gene cassettes. Although the frequency of occurrence of some of the serovars is currently low, their multidrug resistance patterns and presence of class I integrons, which in some cases have not been described, may signify their potential for expansion of the multidrug resistance spectrum and for emerging as foodborne problems.

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