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Antimicrobial Resistance of *Salmonella* Isolates from Swine

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We examined the antimicrobial resistance of 1,257 isolates of 30 serovars of *Salmonella enterica* subsp. *enterica* isolated from swine. Serovars Typhimurium and Typhimurium var. Copenhagen were widespread and were frequently multidrug resistant, with distinct resistance to ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline and to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, respectively.

Nontyphoidal salmonellosis is a major food-borne disease worldwide and is estimated to be responsible for the deaths of more than 500 people each year, with costs of $1.1 billion to $1.5 billion annually in the United States alone (6, 11). In recent years, antimicrobial-resistant *Salmonella* strains have been isolated with increasing frequency. This trend has obvious public health implications, since multidrug resistance (MDR) limits therapeutic options for the treatment of disease in humans and animals. In this report we describe the antimicrobial resistance of *Salmonella* isolates obtained from commercial swine companies.

Samples were collected in three independent studies performed in North Carolina from 1997 through 1998. In Study 1, samples were obtained from two commercial swine production companies that use all in-all out (AIAO) management systems. We twice sampled feces from nine groups of pigs from each company, once at the end of the nursery phase (when the pigs were approximately 10 weeks of age) and again at finisher farms (when the pigs were 26 to 27 weeks of age), on a total of three nursery and nine finisher farms per company. From each group of 640 to 1,000 pigs, 96 fecal samples (weight, approximately 10 g each) were collected from each rectum with a gloved hand at each sampling event. Before the pigs were moved to finishing barns, 10 samples were collected from the disinfected floors by dragging sterile gauze wetted with buffered peptone water (Difco, Detroit, Mich.) over the floors. In this study, a total of 3,456 fecal samples (96 samples each from 18 nursery and 18 finisher group visits) and 180 drag swab specimens were collected. In the second study, two farms that use AIAO and two other farms that use continuous-flow management were chosen. These farms were visited once at the nursery stage and three times at the finishing stage (when the pigs were at 15 and 22 weeks of age and at 48 h before slaughter). At each visit, 1-g fecal samples were collected with sterile swabs from the rectum of each of 60 identified pigs. Prior to slaughter, samples were taken from the transport trucks both before the pigs were loaded and after they were removed. At slaughter, 10 g of cecal tissue and contents and 10 g of mesenteric lymph node were collected. In this study, two replicate samplings were done, for a total of 1,920 fecal samples, 480 cecal samples, and 480 lymph node samples. In the third study, fecal samples were collected from 1,200 pigs from one farm at the nursery and finisher levels. The cecal contents were collected at slaughter. In this study, 168 fecal and 165 cecal isolates were analyzed.

Isolation of *Salmonella* was done by conventional methods (1, 4, 5). Briefly, the samples from studies 1 and 3 were preenriched with buffered peptone water (Becton Dickinson, Franklin Lake, N.J.), and those from study 2 were enriched in Hajna GN and Tetrathionate broth (Difco). The samples in buffered peptone water and Hajna GN were then incubated at 37°C for 24 h; samples in Tetrathionate broth were incubated at 37°C for 48 h. The samples were then transferred to Rappaport Vassilliadis medium (Difco) at 1:100 dilutions and were incubated at 42°C (studies 1 and 3) or 37°C (study 2) for 24 h. All samples were then plated onto Bacto XLT-4 agar base (Difco), and the plates were incubated at 37°C for 24 h. Single colonies were tested for the appropriate reactions on triple sugar iron agar (Difco) and urea agar (Difco). The serovars of the *Salmonella* isolates were determined by the National Veterinary Services Laboratories, Ames, Iowa.

We identified 1,257 *Salmonella enterica* subsp. *enterica* isolates of 30 serovars. The predominant serovars were Typhimurium var. Copenhagen (33.2%), Derby (24.2%), Typhimurium (14.1%), Heidelberg (7.1%), and Infantis (3.9%), together composing 82.5% of all isolates (Table 1). These predominant serovars were also widespread among farms. Serovar Typhimurium var. Copenhagen was isolated from 20 of the 29 farms (71.4%), followed by serovars Derby (16 farms), Typhimurium

TABLE 1. Distribution of *Salmonella* serovars by source

Salmonella serovar	No. (%) of isolates from the following source:					
	Feces	Cecum	Lymph node	Barn	Transport	All sources
Copenhagen	213 (29.8)	100 (28.6)	74 (56.9)	24 (42.9)	6 (75)	417 (33.2)
Derby	166 (23.2)	117 (33.5)	18 (13.8)	3 (5.4)	0	304 (24.2)
Typhimurium	146 (20.4)	15 (4.3)	10 (7.7)	6 (10.7)	0	177 (14.1)
Heidelberg	38 (5.3)	37 (10.6)	9 (6.9)	4 (7.1)	1 (12.5)	89 (7.1)
Infantis	39 (5.3)	7 (2.0)	0	3 (5.4)	0	49 (3.9)
All others	112 (15.7)	73 (20.9)	19 (14.6)	16 (28.6)	1 (12.5)	221 (17.6)
All serovars	714	349	130	56	8	1,257

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TABLE 2. Antimicrobial resistance of *Salmonella* isolates

Antimicrobial	No. (%) resistant isolates of the following serovars:					
	All serovars	Copenhagen	Typhimurium	Derby	Heidelberg	All other serovars
Tetracycline	1,059 (84.2)	400 (95.9)	171 (96.6)	264 (86.8)	86 (96.6)	138 (51.1)
Ampicillin	599 (47.6)	385 (92.3)	170 (96.0)	33 (10.9)	2 (2.2)	9 (4.1)
Piperacillin	462 (36.7)	355 (85.1)	89 (50.3)	8 (2.6)	2 (2.2)	8 (3.6)
Amoxicillin-clavulanic acid	402 (32.0)	350 (83.9)	36 (20.3)	7 (2.3)	2 (2.2)	7 (3.2)
Chloramphenicol	383 (30.5)	329 (78.9)	8 (4.5)	35 (11.5)	5 (5.6)	6 (12.2)
Gentamicin	38 (3.0)	3 (0.7)	1 (0.6)	30 (9.9)	0	4 (1.5)
Cephalothin	18 (1.4)	6 (1.4)	7 (3.9)	1 (0.3)	0	4 (1.5)
Trimethoprim-sulfamethoxazole	15 (1.2)	1 (0.2)	0	6 (1.9)	4 (4.5)	4 (1.8)

(10 farms), Heidelberg (8 farms), and Infantis (5 farms). Serovar Typhimurium var. Copenhagen was also common in all of the sources that we cultured.

We next tested the susceptibilities of the 1,257 isolates to amikacin, amoxicillin-clavulanic acid, ampicillin, cefotaxime, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, piperacillin, tetracycline, and trimethoprim-sulfamethoxazole by MIC testing with the Vitek Jr. automated system (Biomerieux, Hazelwood, Mo.). National Committee for Clinical Laboratory Standards breakpoints were used (7). The susceptibilities of selected isolates to sulfamethoxazole, streptomycin, and kanamycin were determined by Kirby-Bauer disk susceptibility testing on Mueller-Hinton plates by using conventional techniques (8). *Escherichia coli* strains ATCC 25922 and ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control purposes. Isolates for which MICs were intermediate were considered susceptible for the purposes of this study so as not to overstate the extent of resistance. In study 1 a maximum of 15 isolates, chosen by random sampling, per serovar per group of pigs was tested. All isolates from study 2 were tested. Among the isolates from study 3 all isolates obtained from fecal samples were tested, whereas a maximum of five isolates per serovar per slaughter group were tested for isolates of cecal origin.

We found resistance to tetracycline to be common among these isolates, with 84.2% of all isolates being resistant to tetracycline (Table 2). Isolates were commonly resistant to β-lactam antimicrobials as well: ampicillin (47.6%), piperacillin (36.7%), and the combination of amoxicillin and clavulanic acid (32%). Resistance to chloramphenicol, an antimicrobial not used in veterinary medicine for more than a decade, was also prevalent among these isolates (30.5%), suggesting a genetic linkage between chloramphenicol resistance and resistance to other antimicrobials. We found a low frequency of resistance to gentamicin, cephalothin, and trimethoprim-sulfamethoxazole; fewer than 3% of isolates showed resistance to any one of these antimicrobials. We found no resistance to cefotaxime or amikacin. All isolates were also considered susceptible to ciprofloxacin (MICs, $\leq$0.5 μg/ml).

We next examined the frequency of resistance among serovars to determine whether resistance phenotypes were clustered within serovars (Table 2). Resistance to tetracycline was the most widely distributed, with at least 85% of isolates from the four most common serovars being resistant to that antimicrobial. For the other antimicrobials, however, only one or two of the serovars accounted for a great majority of the resistant isolates. For example, 47.6% of all isolates were ampicillin resistant, but serovars Typhimurium and Typhimurium var. Copenhagen together constituted 92.6% (555 of 599) of all ampicillin-resistant isolates. Similarly, 32% of all isolates were resistant to amoxicillin-clavulanic acid, but 83.9% of serovar Typhimurium var. Copenhagen isolates were resistant (comprising 87% of all the amoxicillin-clavulanic acid-resistant isolates). Also, chloramphenicol resistance was predominantly found among serovar Typhimurium var. Copenhagen isolates; 30.5% of all isolates were resistant to this antimicrobial, but 87% of these were serovar Typhimurium var. Copenhagen (83.9% of all serovar Typhimurium var. Copenhagen isolates). Among antimicrobials to which resistance was less common, differences among serovars were also seen; only 3% of all isolates were gentamicin resistant, but 79% of these (30 of 38) were serovar Derby. These results show that the most frequently isolated serovars were also most likely to express antimicrobial resistance factors.

We next determined the frequency of MDR, identified the common patterns of resistance, and determined whether these patterns could be correlated with specific serovars. We found that 625 (49.7%) of our isolates were resistant to two or more of the antimicrobials tested (Table 3). However, the majority of these isolates were serovars Typhimurium and Typhimu-

TABLE 3. Distribution of predominant resistance patterns among major serovars

Resistance pattern[a]	No. (%) of isolates of the following serovars with the resistance pattern:					
	All isolates	Copenhagen	Typhimurium	Derby	Heidelberg	All other serovars
No resistance	177 (14.1)	10 (2.4)	4 (2.3)	33 (10.9)	1 (1.1)	129 (47.8)
Te alone	455 (36.2)	21 (5.0)	3 (1.7)	230 (75.7)	81 (91.0)	120 (44.4)
AmTe	91 (7.2)	21 (5.0)	69 (39.0)	1 (0.3)	0	0
AmPiTe	63 (5.0)	10 (2.4)	51 (28.8)	0	0	2 (0.7)
AmAxPiTe	36 (2.9)	9 (2.2)	22 (12.4)	0	0	5 (1.8)
AmAxCmPiTe	324 (25.8)	319 (76.5)	2 (1.1)	1 (0.3)	2 (2.2)	0
All MDR patterns[b]	625 (49.7)	386 (92.6)	170 (96)	41 (13.5)	7 (7.9)	21 (7.8)

[a] Am, ampicillin; Ax; amoxicillin-clavulanic acid; Cm, chloramphenicol; Pi, piperacillin; Te, tetracycline.
[b] MDR is defined as resistance to two or more of the antimicrobials tested.

rium var. Copenhagen. Among the serovar Typhimurium isolates, 96% were MDR, and a total of 80% were resistant to ampicillin and tetracycline, alone or in combination with resistance to piperacillin or amoxicillin-clavulanic acid. The majority of serovar Typhimurium var. Copenhagen isolates (76.5%) had a single antimicrobial resistance pattern: ampicillin, amoxicillin-clavulanic acid, chloramphenicol, piperacillin, and tetracycline. This pattern was detected in isolates derived from 20 of the 29 farms, suggesting that these MDR isolates were widespread among the farms studied. Similarly, MDR serovar Typhimurium isolates were obtained from 10 farms. We also found that serovar Typhimurium and Typhimurium var. Copenhagen isolates had the greatest diversity of resistance patterns, with 16 different patterns each. Thus, 89% of our MDR isolates were either serovar Typhimurium or serovar Copenhagen, and these were some of the most widespread.

Salmonella of the MDR DT104 phage type have a characteristic pentadrug resistance pattern, with resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (AmCmStSuTe) (10). To determine whether phage type DT104 might be present among our isolates, we screened those that were resistant to at least ampicillin, chloramphenicol, and tetracycline (AmCmTe) for resistance to sulfamethoxazole and streptomycin. As a further test, we examined isolates for susceptibility to kanamycin, an antimicrobial to which resistance has been reported among *Salmonella* strains but not one to which DT104 strains are commonly resistant. We tested 99 AmCmTe serovar Typhimurium var. Copenhagen isolates, as well as 6 serovar Typhimurium and 14 serovar Derby isolates, and found that 98 of 99 serovar Typhimurium var. Copenhagen isolates, all serovar Typhimurium isolates, and 11 of 14 serovar Derby isolates also exhibited resistance to streptomycin and sulfamethoxazole. We next tested representatives of the AmCmStSuTe isolates for the presence of the resistance alleles found in DT104 isolates using multiplex PCR (3). DT104 carries its five resistance factors on two adjacent integrons, harboring *aadA2*, *cmlA*, and *tetA-tetR* (which encode streptomycin, chloramphenicol, and tetracycline resistance, respectively) and expressing ampicillin resistance by means of $bla_{\text{PSE-1}}$ (2). As is common for this class of integron, these also encode sulfonamide resistance (9). DNAs were extracted from selected isolates with a DNAeasy kit (Qiagen, Valencia, Calif.), and approximately 100 ng of template DNA was used for each reaction. Multiplex PCRs contained primers for $bla_{\text{PSE-1}}$, which encodes ampicillin resistance, and primers for physically linked *cmlA* and *tetR* genes, which encode chloramphenicol and tetracycline resistances, respectively, as well as primers for the *Salmonella*-specific genes *sipB* and *sipC*. Isolates with the $bla_{\text{PSE-1}}$ (150 bp) and the *cmlA-tetR* (280 bp) amplicons were tentatively identified as phage type DT104 strains. We also examined isolates for the presence of a second β-lactamase gene (bla_{TEM}) in a separate PCR (3). We found that all of the serovar Typhimurium var. Copenhagen isolates with the AmCmStSuTe resistance pattern that we tested (32 of 32 isolates derived from 14 farms) showed the pattern commonly seen in phage type DT104 isolates (Table 4). Interestingly, all of these isolates were also resistant to amoxicillin-clavulanic acid, a characteristic found among only 24.5% of ampicillin-resistant isolates of all other serovars. In contrast, of the six serovar Typhimurium isolates with the AmCmStSuTe resistance pattern, only one showed the presence of the resistance-encoding alleles commonly found in phage type DT104 isolates. The single serovar Derby isolate with this resistance pattern also did not have the resistance-encoding alleles of DT104 isolates, showing that the AmCmStSuTe resistance pattern can be expressed by types other than DT104.

TABLE 4. Characterization of resistance determinants among MDR strains

Serovar and resistance pattern[a]	No. of isolates	No. of isolates for which the following resistance allele was amplified:	
		cmlA-tetR and $bla_{\text{PSE-1}}$	bla_{TEM}
Copenhagen			
AmCmStSuTe	32	32	0
AmKmStSuTe	22	0	22
Typhimurium			
AmCmStSuTe	6	1	4
AmKmStSuTe	29	0	29
Derby			
AmCmStSuTe	1	0	0
AmCmGmKmStSuTe	10	0	10

[a] Am, ampicillin; Cm, chloramphenicol; St, streptomycin; Su, sulfamethoxazole; Te, tetracycline; Km, kanamycin; Gm, gentamicin.

We also examined MDR isolates with patterns that included resistance to kanamycin. We found a number of isolates with resistance to the combination of ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline. This pattern was the predominant pentadrug resistance pattern among the serovar Typhimurium isolates tested and was second only to the AmCmStSuTe pattern among the pentadrug-resistant serovar Typhimurium var. Copenhagen isolates. Of the 100 isolates for which data are presented in Table 4, 51 had this pattern, with 22 being serovar Typhimurium var. Copenhagen isolates and 29 being serovar Typhimurium isolates. All of these isolates were also shown to encode ampicillin resistance by means of the bla_{TEM} gene rather than the $bla_{\text{PSE-1}}$ gene of phage type DT104 isolates. We also found 10 serovar Derby isolates that were resistant to these five antimicrobials but that were additionally resistant to chloramphenicol and gentamicin (Table 4). These isolates were derived from one farm but were collected at three different times from the same group of pigs. The presence of these MDR patterns shows that MDR strains other than phage type DT104 strains exist among the most common *Salmonella* serovars in commercial swine herds.

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