

Antimicrobial Resistance Genes Associated with *Salmonella enterica* Serovar Newport Isolates from Food Animals[∇]

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***Salmonella enterica* serotype Newport is an important cause of salmonellosis, with strains increasingly being resistant to multiple antimicrobial agents. The increase is associated with the acquisition of multiple resistance genes. This study characterizes the genetic basis of resistance of serotype Newport isolates collected from veterinary sources by PCR and DNA sequencing analysis.**

Salmonellosis is a significant public health concern in the United States. From 1997 to 2003, the number of laboratory-confirmed *Salmonella enterica* serovar Newport infections reported to CDC increased from 1,584 (4.6% of all reported *Salmonella* infections) to 4,000 (10.3%) (5). Over the same period, the percentage of multidrug-resistant serovar Newport isolates increased nearly fivefold (6). Therefore, the increasing number of serovar Newport infections appears to be associated with the emergence and rapid dissemination of multidrug-resistant (MDR) strains of serovar Newport (18). Multidrug resistance is often associated with mobile genetic elements (i.e., plasmids and transposons) that encode multiple specific resistance genes (1, 12, 13, 17, 19).

The objective of this study was to further characterize the genetic basis of multiantimicrobial resistance in *Salmonella* serovar Newport isolates collected from food animals in the United States. These isolates were previously characterized by pulsed-field gel electrophoresis (PFGE), by antimicrobial susceptibility testing, and for their ability to transfer resistance-conferring plasmids from MDR strains to susceptible ones (19). The subset of isolates included in the present study represents strains isolated from chicken, swine, turkey, and cattle from 23 states in the United States (Fig. 1). The susceptibilities of the isolates to the following agents were determined: amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole. Isolates were screened for the presence of 19 genes associated with resistance to 12 of the antibiotics in which resistance was detected previously (no resistance to amikacin, ciprofloxacin, or nalidixic acid was detected) (19). Resistance genes were detected by PCR with the primers shown in Table 1. The reactions were repeated to confirm the initial results, and representative amplification products were sequenced to verify the identities of the PCR products (7). Additionally, plasmids were

isolated (16) and analyzed to determine whether there was commonality between particular plasmids and the resistance observed.

Overall, when resistance was seen, a corresponding resistance gene was detected 96.6% of the time (Fig. 1). Likewise, nearly all of the MDR strains contained large plasmids (≥ 75 kb), while the pansusceptible strains did not (Fig. 1). Nineteen isolates shared resistance to amoxicillin-clavulanic acid, ampicillin, cephalothin, cefoxitin, and ceftiofur and reduced susceptibility (MIC of 16 or 32 $\mu\text{g/ml}$) or resistance to ceftriaxone. Each of these isolates was positive for *bla*_{CMY}, which is most commonly associated with ceftiofur and ceftriaxone resistance in *Salmonella* strains. A single isolate was resistant to ceftriaxone and was positive for both *bla*_{CMY} and *bla*_{TEM}. Five additional isolates were positive for *bla*_{TEM}. Two of these five isolates were resistant to ampicillin and cephalothin; one was resistant to ampicillin, amoxicillin-clavulanic acid, and ceftiofur; and two were susceptible to all agents tested. Eleven isolates were resistant to kanamycin, and each isolate contained *aphAI-IAB*. Twenty-three isolates demonstrated resistance to streptomycin, and of these isolates, 21 were positive for *strA*, 20 were positive for *aadA1*, 11 were positive for *strB*, and 7 were positive for *aadA2*. Five of the seven gentamicin-resistant isolates carried *aacC*, while none contained *aadB*. Both genes have been associated with gentamicin resistance in *Salmonella* serovar Newport isolates (13, 17). Three isolates contained *aacC* but were not resistant to gentamicin. Even though they did not meet the threshold for resistance, two had decreased susceptibility (MIC of 1 and 8 $\mu\text{g/ml}$) compared to the level of susceptibility of the majority of susceptible isolates (MIC ≤ 0.25 $\mu\text{g/ml}$), likely indicating that the enzymes displayed variable levels of activity in these strains. Twenty-four isolates were resistant to sulfisoxazole; all but one contained *sul1*, *sul2*, and/or *sul3*. Five isolates were resistant to trimethoprim-sulfamethoxazole, and three of these isolates carried the *dfrXII* resistance gene in a class 1 integron (19). The remaining two isolates contained class 1 integrons that lacked *dfr* genes. *Salmonella* strains containing *dfrXII* have predominantly been reported in Europe and Asia, with few reports of such strains in the United States (2, 4, 9, 12, 15). Conversely, there have been reports of *dfrXII*-associated resistance in *Escherichia coli* isolates from the United States (14), indicating that the genes are

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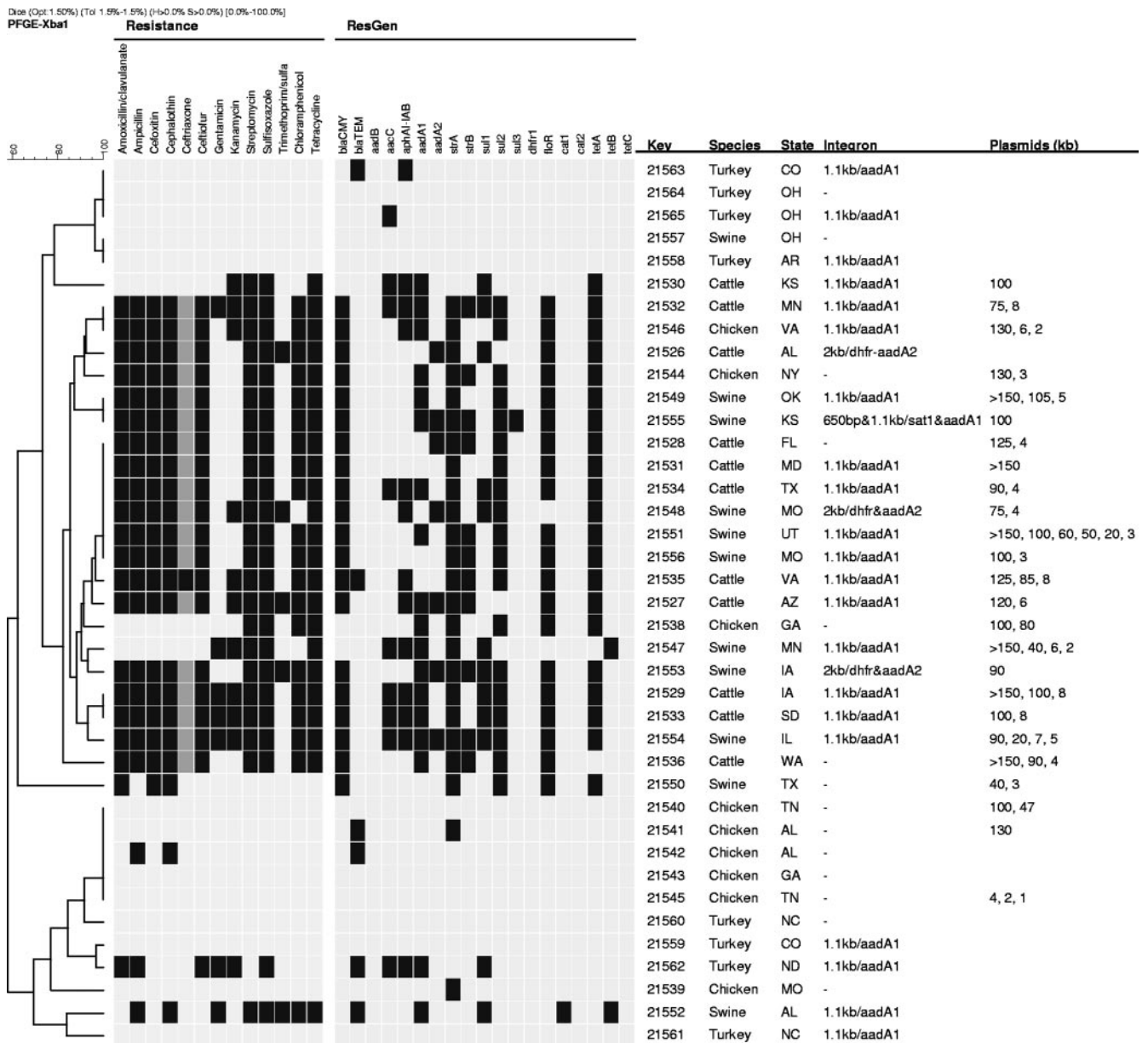


FIG. 1. Antimicrobial susceptibility testing and antimicrobial resistance gene detection results for the *Salmonella* serovar Newport isolates characterized in the study. The isolates are arranged by a PFGE profile similarity dendrogram (calculated in reference 19) and are described by culture number (Key), animal of origin (Species), state of origin (State), the sizes and resistance gene inserts in the class 1 integrons (Integron), and the sizes of plasmids isolated from each strain (Plasmids [kb]). For the susceptibility testing results (Resistance), a black box indicates resistance, a light gray box susceptibility, and a darker gray box reduced susceptibility to ceftriaxone. For resistance gene detection (ResGen), a black box indicates that the gene was detected by PCR, and a light gray box indicates the absence of the gene.

present in microbial populations. Twenty isolates were resistant to chloramphenicol; 19 of these isolates were positive for *floR*, while the other isolate contained *cat1*. Additionally, 23 isolates were resistant to tetracycline. Twenty isolates contained *tetA*, two had *tetB*, and one isolate lacked the *tet* genes that we screened for. In cases in which an associated resistance gene was not identified, resistance was likely due to another member of the resistance gene class.

In most cases, our findings were similar to those reported previously (11, 13); however, the detection of *aacC*-associated

gentamicin resistance is in contrast to the findings from other countries, where *aadB* was the predominant factor associated with gentamicin resistance in *Salmonella* serovar Newport (16, 19). Thus, our results, along with those of Welch et al. (17), indicate that *aacC* may play a larger role than *aadB* in gentamicin resistance in serovar Newport isolates from the United States. Additionally, the only isolate resistant to ceftriaxone contained both *bla*_{TEM} and *bla*_{CMY}, which likely indicates synergism among the β-lactamases. Our results also indicated that some isolates with integrons containing *aadA1* were suscepti-

TABLE 1. PCR primers and gene targets for antimicrobial resistance

Gene	Primer ^a	Nucleotide sequence (5' to 3')	Annealing temp (°C) ^b	Resistance mechanism	Resistance ^c	Reference or source
<i>aadA1</i>	F R	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	54	Aminoglycoside adenylyltransferase	STR	13
<i>aadA2</i>	F R	TGTTGGTTACTGTGGCCGTA GATCTCGCCTTTCACAAAGC	62	Aminoglycoside adenylyltransferase	STR	13
<i>aadB</i>	F R	GAGCGAAATCTGCCGCTCTGG CTGTTACAACGGACTGGCCGC	61	Aminoglycoside adenylyltransferase	GEN	13
<i>aacC</i>	F R	GGCGGATCAACGAATTTATCCGA CCATTTCGATGCCGAAGGAAACGAT	58	Aminoglycoside acetyltransferase	GEN	This study
<i>aphAI-LAB</i>	F R	AAACGTCTTGCTCGAGGC CAAACCGTTATTCATTTCGTGA	55	Aminoglycoside phosphoryltransferase	KAN	13
<i>bla_{CMY}</i>	F R	GACAGCCTCTCTTTCTCCACA TGGAACGAAGGCTACGTA	50	β-Lactamase	AMP	13
<i>bla_{TEM}</i>	F R	CATTTCCGTGTGCGCCCTTAT TCCATAGTTGCGCTGACTCCC	55	β-Lactamase	AMP	13
<i>cat1</i>	F R	CCTATAACCAGACCGTTCAG TCACAGACGGCATGATGAAC	56	Chloramphenicol acetyltransferase	CHL	13
<i>cat2</i>	F R	CCGGATTGACCTGAATACCT TCACATACTGCATGATGAAC	56	Chloramphenicol acetyltransferase	CHL	13
<i>dfrI</i>	F R	GTGAAACTATCACTAATGGTAGCT ACCCTTTTGCCAGATTTGGTAACT	54	Dihydrofolate reductase inhibitor	TMP	13
<i>floR</i>	F R	AACCCGCCCTCTGGATCAAGTCAA CAAATCACGGGCCACGCTGTATC	60	Efflux	CHL	13
<i>strA</i>	F R	AGCAGAGCGCGCCTTCGCTC CCAAAGCCCACTTCACCGAC	59	Aminoglycoside phosphoryltransferase	STR	13
<i>strB</i>	F R	ATCGTCAAGGGATTGAAACC GGATCGTAGAACATATTGGC	49	Aminoglycoside phosphoryltransferase	STR	3
<i>sul1</i>	F R	TCACCGAGGACTCCTTCTTC AATATCGGGATAGAGCGCAG	60	Dihydropteroate synthase inhibitor	SUL	13
<i>sul2</i>	F R	CGGTCCGGCATCCAGCAATCC CGAGAGCCACGACCGCGCC	64	Dihydropteroate synthase inhibitor	SUL	13
<i>sul3</i>	F R	GAGCAAGATTTTTGGAATCG CATCTGCAGCTAACCTAGGGCTTGGGA	51	Dihydropteroate synthase inhibitor	SUL	3
<i>tetA</i>	F R	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	55	Efflux	TET	8
<i>tetB</i>	F R	TTGGTTAGGGGCAAGTTTTG GTAATGGGCAATAACACCG	53	Efflux	TET	8
<i>tetC</i>	F R	CTTGAGAGCCTTCAACCCAG ATGGTCGTCATCTACCTGCC	56	Efflux	TET	8
<i>intA</i>	F R	GGCATCCAAGCAGCAAGC AAGCAGACTTGACCTGAT	50	Class 1 integron		13
<i>intB</i>	F R	ATCGCAATAGTTGGCGAGT GCAAGGCGGAAACCCGCGCC	53	<i>qacEΔ-sulI</i> of class 1 integron		13

^a F, forward; R, reverse.^b Annealing temperature for each PCR.^c The gene encodes resistance to ampicillin (AMP), chloramphenicol (CHL), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), sulfisoxazole (SUL), tetracycline (TET), and trimethoprim (TMP).

ble to streptomycin. Previous reports have shown that silent integron-borne *aadA* genes in *Salmonella* strains may be expressed when they are transferred to a new host by conjugation (20). All of our streptomycin-susceptible isolates that contained class 1 integrons with *aadA1* were isolated from turkeys from multiple states and were spread among divergent PFGE clusters (19), which may indicate that these integrons are disseminated among serovar Newport isolates in turkey flocks. There were some additional differences among the *Salmonella* isolates from different animal sources, including the fact that *tetB* was detected only in our swine isolates and that one of these isolates was also the only strain with *cat1*-associated chloramphenicol resistance.

Overall, cattle and swine isolates demonstrated resistance to the greatest number of antimicrobial agents, which paralleled the results from NARMS from 2001 and 2002 (6). When the PFGE profiles of the isolates were compared, almost all MDR isolates were clustered together (19). These related strains may share a higher capacity to acquire resistance plasmids, thus contributing to the rapid rise of MDR *Salmonella* serovar Newport isolates over the last decade as important causes of human salmonellosis. The results of this study provide further information on the contribution of specific resistance genes to MDR in serovar Newport strains.

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