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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 μg/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 μg/ml) compared to the level of susceptibility of the majority of susceptible isolates (MIC ≤ 0.25 μg/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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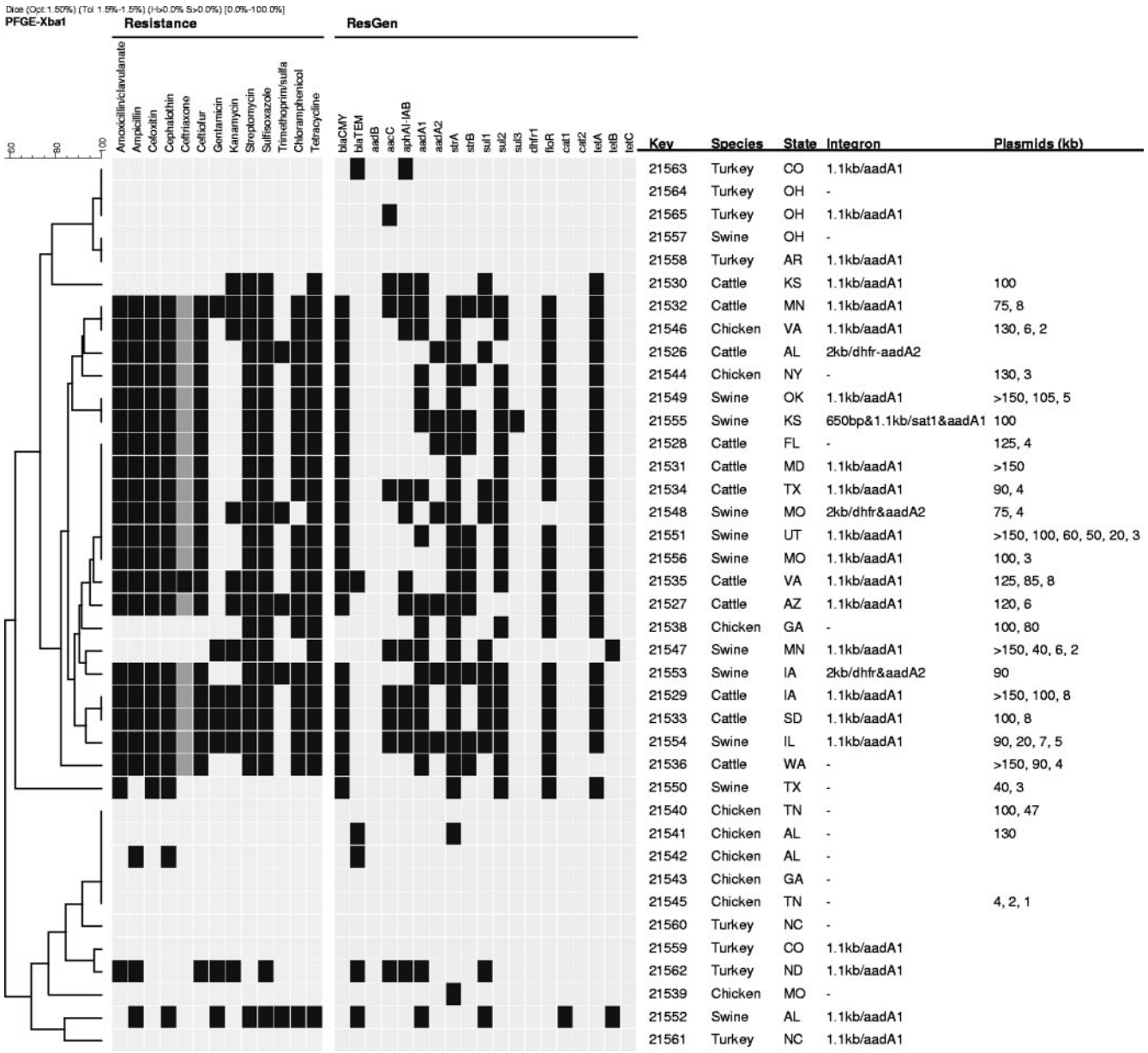

FIG. 1. Antimicrobial susceptibility testing and antimicrobial resistance gene detection results for the *Salmonella* serovar Newport isolates characterized in the study. The isolates are arranged by a PFGE profile similarity dendrogram (calculated in reference 19) and are described by culture number (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
aadA1	F	TATCAGAGGTAGTTGGCGTCAT	54	Aminoglycoside adenyltransferase	STR	13
	R	GTTCCATAGCGTTAAGGTTTCATT				
aadA2	F	TGTTGGTTACTGTGGCCGTA	62	Aminoglycoside adenyltransferase	STR	13
	R	GATCTCGCCTTTCACAAAGC				
aadB	F	GAGCGAAATCTGCCGCTCTGG	61	Aminoglycoside adenyltransferase	GEN	13
	R	CTGTTACAACGGACTGGCCGC				
aacC	F	GGCGCGATCAACGAATTTATCCGA	58	Aminoglycoside acetyltransferase	GEN	This study
	R	CCATTCGATGCCGAAGGAAACGAT				
aphAI-IAB	F	AAACGTCTTGCTCGAGGC	55	Aminoglycoside phosphoryltranferase	KAN	13
	R	CAAACCGTTATTCATTCGTGA				
bla_{CMY}	F	GACAGCCTCTCTTTCTCCACA	50	β-Lactamase	AMP	13
	R	TGGAACGAAGGCTACGTA				
bla_{TEM}	F	CATTTCCGTGTCGCCCTTAT	55	β-Lactamase	AMP	13
	R	TCCATAGTTGCCTGACTCCC				
cat1	F	CCTATAACCAGACCGTTCAG	56	Chlorampenicol acetyltransferase	CHL	13
	R	TCACAGACGGCATGATGAAC				
cat2	F	CCGGATTGACCTGAATACCT	56	Chlorampenicol acetyltransferase	CHL	13
	R	TCACATACTGCATGATGAAC				
dfrI	F	GTGAAACTATCACTAATGGTAGCT	54	Dihydrofloate reductase inhibitor	TMP	13
	R	ACCCTTTTGCCAGATTTGGTAACT				
floR	F	AACCCGCCCTCTGGATCAAGTCAA	60	Efflux	CHL	13
	R	CAAATCACGGGCCACGCTGTATC				
strA	F	AGCAGAGCGCGCCTTCGCTC	59	Aminoglycoside phosphoryltranferase	STR	13
	R	CCAAAGCCCACTTCACCGAC				
strB	F	ATCGTCAAGGGATTGAAACC	49	Aminoglycoside phosphoryltranferase	STR	3
	R	GGATCGTAGAACATATTGGC				
sul1	F	TCACCGAGGACTCCTTCTTC	60	Dihydropteroate synthase inhibitor	SUL	13
	R	AATATCGGGATAGAGCGCAG				
sul2	F	CGGTCCGGCATCCAGCAATCC	64	Dihydropteroate synthase inhibitor	SUL	13
	R	CGAGAGCCACGACCGCGCC				
sul3	F	GAGCAAGATTTTTGGAATCG	51	Dihydropteroate synthase inhibitor	SUL	3
	R	CATCTGCAGCTAACCTAGGGCTTGGA				
tetA	F	GCTACATCCTGCTTGCCTTC	55	Efflux	TET	8
	R	CATAGATCGCCGTGAAGAGG				
tetB	F	TTGGTTAGGGGCAAGTTTTG	53	Efflux	TET	8
	R	GTAATGGGCCAATAACACCG				
tetC	F	CTTGAGAGCCTTCAACCCAG	56	Efflux	TET	8
	R	ATGGTCGTCATCTACCTGCC				
intA	F	GGCATCCAAGCAGCAAGC	50	Class 1 integron		13
	R	AAGCAGACTTGACCTGAT				
intB	F	ATCGCAATAGTTGGCGAGT	53	*qacE*Δ-*sul1*of class 1 integron		13
	R	GCAAGGCGGAAACCCGCGCC				

[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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REFERENCES

1. **Aarts, H. J., K. S. Boumedine, X. Nesme, and A. Cloeckaert.** 2001. Molecular tools for the characterisation of antibiotic-resistant bacteria. Vet. Res. **32:** 363–380.
2. **Antunes, P., J. Machado, and L. Peixe.** 2007. Dissemination of *sul3*-containing elements linked to class 1 integrons with an unusual 3′ conserved sequence region among *Salmonella* isolates. Antimicrob. Agents Chemother. **51:**1545–1548.
3. **Boerlin, P., R. Travis, C. L. Gyles, R. Reid-Smith, N. Janecko, H. Lim, V. Nicholson, S. A. McEwen, R. Friendship, and M. Archambault.** 2005. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. Appl. Environ. Microbiol. **71:**6753–6761.
4. **Cabrera, R., J. Ruiz, F. Marco, I. Oliveira, M. Arroyo, A. Aladuena, M. A. Usera, M. T. Jimenez De Anta, J. Gascon, and J. Vila.** 2004. Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing traveler's diarrhea. Antimicrob. Agents Chemother. **48:**3934–3939.
5. **Centers for Disease Control and Prevention.** 2005. *Salmonella* surveillance: annual summary, 2004. Centers for Disease Control and Prevention, Atlanta, GA.
6. **FDA.** 2006. National antimicrobial resistance monitoring system—enteric bacteria (NARMS): 2003 executive report. FDA, U.S. Department of Health and Human Services, Washington, DC.
7. **Foley, S. L., D. G. White, P. F. McDermott, R. D. Walker, B. Rhodes, P. J. Fedorka-Cray, S. Simjee, and S. Zhao.** 2006. Comparison of subtyping methods for differentiating *Salmonella enterica* serovar Typhimurium isolates obtained from food animal sources. J. Clin. Microbiol. **44:**3569–3577.
8. **Fonseca, E. L., O. L. Mykytczuk, M. D. Asensi, E. M. Reis, L. R. Ferraz, F. L. Paula, L. K. Ng, and D. P. Rodrigues.** 2006. Clonality and antimicrobial resistance gene profiles of multidrug-resistant *Salmonella enterica* serovar Infantis isolates from four public hospitals in Rio de Janeiro, Brazil. J. Clin. Microbiol. **44:**2767–2772.
9. **Hsu, S. C., T. H. Chiu, J. C. Pang, C. H. Hsuan-Yuan, G. N. Chang, and H. Y. Tsen.** 2006. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. Int. J. Antimicrob. Agents. **27:**383–391.
10. Reference deleted.
11. **Poppe, C., L. Martin, A. Muckle, M. Archambault, S. McEwen, and E. Weir.** 2006. Characterization of antimicrobial resistance of *Salmonella* Newport isolated from animals, the environment, and animal food products in Canada. Can. J. Vet. Res. **70:**105–114.
12. **Poppe, C., L. C. Martin, C. L. Gyles, R. Reid-Smith, P. Boerlin, S. A. McEwen, J. F. Prescott, and K. R. Forward.** 2005. Acquisition of resistance to extended-spectrum cephalosporins by *Salmonella enterica* subsp. *enterica* serovar Newport and Escherichia coli in the turkey poult intestinal tract. Appl. Environ Microbiol. **71:**1184–1192.
13. **Randall, L. P., S. W. Cooles, M. K. Osborn, L. J. Piddock, and M. J. Woodward.** 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J. Antimicrob. Chemother. **53:**208–216.
14. **Singh, R., C. M. Schroeder, J. Meng, D. G. White, P. F. McDermott, D. D. Wagner, H. Yang, S. Simjee, C. Debroy, R. D. Walker, and S. Zhao.** 2005. Identification of antimicrobial resistance and class 1 integrons in Shiga toxin-producing *Escherichia coli* recovered from humans and food animals. J. Antimicrob. Chemother. **56:**216–219.
15. **Vo, A. T. T., E. van Duijkeren, A. C. Fluit, and W. Gaastra.** 2 April 2007. Antimicrobial resistance, class 1 integrons and a novel variant of genomic island 1 in *Salmonella* isolates from Vietnam. Antimicrob. Agents Chemother. doi:10.1128/AAC.01093-06.
16. **Wang, Z., and T. G. Rossman.** 1994. Large-scale supercoiled plasmid preparation by acidic phenol extraction. BioTechniques **16:**460–463.
17. **Welch, T. J., W. F. Fricke, P. F. McDermott, D. G. White, M. L. Rosso, D. A. Rasko, M. K. Mammel, M. Eppinger, M. J. Rosovitz, D. Wagner, L. Rahalison, J. E. Leclerc, J. M. Hinshaw, L. E. Lindler, T. A. Cebula, E. Carniel, and J. Ravel.** 2007. Multiple antimicrobial resistance in plague: an emerging public health risk. PLoS ONE **2:**e309.
18. **Zansky, S., B. Wallace, D. Schoonmaker-Bopp, P. Smith, F. Ramsey, J. Painter, A. Gupta, P. Kalluri, and S. Noviello.** 2002. From the Centers for Disease Control and Prevention. Outbreak of multi-drug resistant *Salmonella* Newport—United States, January–April 2002. JAMA **288:**951–953.
19. **Zhao, S., S. Qaiyumi, S. Friedman, R. Singh, S. L. Foley, D. G. White, P. F. McDermott, T. Donkar, C. Bolin, S. Munro, E. J. Baron, and R. D. Walker.** 2003. Characterization of *Salmonella enterica* serotype Newport isolated from humans and food animals. J. Clin. Microbiol. **41:**5366–5371.
20. **Zhao, S., D. G. White, B. Ge, S. Ayers, S. Friedman, L. English, D. Wagner, S. Gaines, and J. Meng.** 2001. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl. Environ. Microbiol. **67:**1558–1564.