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Antimicrobial Resistance in *Escherichia coli* Isolates from Swine and Wild Small Mammals in the Proximity of Swine Farms and in Natural Environments in Ontario, Canada▽

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Wild animals not normally exposed to antimicrobial agents can acquire antimicrobial agent-resistant bacteria through contact with humans and domestic animals and through the environment. In this study we assessed the frequency of antimicrobial resistance in generic *Escherichia coli* isolates from wild small mammals (mice, voles, and shrews) and the effect of their habitat (farm or natural area) on antimicrobial resistance. Additionally, we compared the types and frequency of antimicrobial resistance in *E. coli* isolates from swine on the same farms from which wild small mammals were collected. Animals residing in the vicinity of farms were five times more likely to carry *E. coli* isolates with tetracycline resistance determinants than animals living in natural areas; resistance to tetracycline was also the most frequently observed resistance in isolates recovered from swine (83%). Our results suggest that *E. coli* isolates from wild small mammals living on farms have higher rates of resistance and are more frequently multiresistant than *E. coli* isolates from environments, such as natural areas, that are less impacted by human and agricultural activities. No *Salmonella* isolates were recovered from any of the wild small mammal feces. This study suggests that close proximity to food animal agriculture increases the likelihood that *E. coli* isolates from wild animals are resistant to some antimicrobials, possibly due to exposure to resistant *E. coli* isolates from livestock, to the resistance genes of these isolates, or to antimicrobials through contact with animal feed.

The impact of antimicrobial resistance (AMR) in bacteria from farm animals on humans and the environment is a growing concern. Wild animals are not normally exposed to antimicrobial agents, but through direct and indirect interactions with humans, food, and domestic animals, they may come in contact with resistant bacteria. Such contact is believed to be responsible for the dissemination of resistant bacteria and horizontal transfer of AMR genes among bacteria from wild animal populations. Several studies have supported this hypothesis by demonstrating that the AMR rates are higher among animals living close to humans and agricultural areas than among wild animals residing in more isolated regions (2, 5, 35). Bacterial isolates obtained from wildlife whose habitat is utilized by humans are more likely to be resistant to antimicrobials than isolates in more pristine areas farther from humans or agricultural infrastructure (10, 26). The acquisition of resistance genes in wild and free-ranging populations is a concern as this may create an environmental reservoir of AMR in animals which usually have no contact with "man-made" antimicrobials (5, 14).

Resistance to a variety of antimicrobials used in human and veterinary medicine, including resistance to streptomycin, ampicillin, tetracycline, sulfonamides, kanamycin, and gentamicin, has been detected in bacteria obtained from wildlife (2, 5, 25, 32, 36). Most of the previous literature on AMR in wildlife has focused on resistance phenotypes. To our knowledge, only very limited AMR genotyping has been performed for bacteria from wild animals (5, 7). The resistance genes that have been identified in bacteria from wild animal populations include bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, *tet*(A), *tet*(B), *aadA*, *sul1*, and *sul2* (7, 26). Genotyping is important because resistance to a specific antimicrobial agent can be caused by many different determinants which may have distinct epidemiologies and different associations with animal or bacterial species. Furthermore, resistance genes are often linked together on mobile genetic elements, such as plasmids. Through genotyping we can determine the genes which are responsible for resistance in wild populations, determine genetic associations, and possibly link the origin of the genes to an external source, such as humans or animal species.

The objective of this study was to determine the impact that agriculture may have on the occurrence of antimicrobial resistance in common wild small mammals living in Ontario, Canada. First, we compared AMR determinants in bacteria isolated from wild small mammals (mice, voles, and shrews) living in natural areas (presumed to have very little or no exposure to AMR or antimicrobials) with AMR determinants in bacteria isolated from wild small mammals found on swine farms (presumed to be exposed to AMR and antimicrobials). Second, we assessed whether the AMR profiles and resistance genes of *Escherichia coli* isolates from small mammals living on swine farms were similar to those of *E. coli* isolates from the swine themselves, which would suggest that there was potential transfer of resistant bacteria or resistance determinants between the swine and wild animals.

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TABLE 1. Locations, sampling dates, and species of wild mammals trapped in natural areas and in the vicinity of swine farms in this study

Site[a]	Sampling dates[b]	No. of trap nights[c]	No. of animals with *E. coli*/no. of animals trapped				
			Total	*Peromyscus* sp.	*Microtus pennsylvanicus*	*Blarina brevicauda*	*Mus musculus*
Natural 1	20 to 22 June	240	9/15	9/15			
Farm 1	27 to 29 June	240	2/4	1/1	0/1		1/2
Natural 2	13 to 15 November	240	1/2	0/1		1/1	
Farm 2	13 to 15 June	240	6/14	5/11		0/2	1/1
Natural 3	26 to 27 October	160	3/4	2/3		1/1	
Farm 3	19 to 21 June	240	6/11	2/3	1/3		3/5
Natural 4	26, 27, and 29 June	240	2/8	2/6	0/2		
Farm 4	8 to 10 November	240	1/3	0/1		1/2	
Natural 5	4 to 6 July	480	5/16	4/12		1/4	
Farm 5	18 to 20 October	240	7/9	2/4		1/1	4/4
Total		2,560	42/86	27/57	1/6	5/11	9/12

[a] Natural, conservation areas with minimal impact of human or farming activities; Farm, vicinity and premises of swine farms.
[b] All sampling was done in 2007.
[c] The number of trap nights was determined by multiplying the number of traps set each night by the number of nights of trapping at the site.

MATERIALS AND METHODS

Trapping and sampling. All procedures for trapping and handling wild small mammals were approved by the animal care committee of the University of Guelph (University of Guelph Animal Utilization Protocol 07R042). Small mammals with limited home ranges that were likely to be present in both agricultural and natural areas were targeted in this study (mice, voles and shrews). Animals were live trapped using Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, FL) in five natural areas and on five nearby farms in the Grand River Watershed (43°35′N, 80°15′W; Ontario, Canada) from June to November, 2007 (Table 1). The areas of the natural sites ranged from approximately 32 to 5,915 ha. We attempted to pair farms and natural sites, and the members of most pairs were within 5 km of each other; the only exception was natural area 5 and farm 5, which were approximately 35 km apart. Eight 10-trap transects with 10-m spacing were set at most site (80 traps) for three nights; the only exceptions were natural area 3, where traps were set for only two nights because of disturbance by raccoons, and natural area 5, where 160 traps were set for three nights (Table 1). The transects in each natural area were at least 100 m from the edge of the area at sites at which there was little or no evidence of human activity. The transects on farms were placed around barns and other buildings, around feed and manure storage areas, and along edges of vegetation.

Target animals were anesthetized using halothane (MTC Pharmaceuticals, Ontario, Canada) prior to handling and were euthanized using an overdose of halothane. Blood was collected via cardiac puncture and was shown to be negative for hantavirus by PCR or serology at the National Microbiology Laboratory (Winnipeg, Manitoba, Canada). Appropriate precautions (23) were taken by workers to avoid exposure to zoonotic agents. Each animal captured was identified to the genus level in the case of *Peromyscus* (either *Peromyscus maniculatus* or *Peromyscus leucopus*) or to the species level for all other animals. The large intestine was removed from each carcass, and the intestinal contents were aseptically extruded into 5 ml brain heart infusion (BHI) broth (Becton Dickinson, Oakville, Ontario, Canada) containing 20% glycerol as a cryoprotectant for subsequent *E. coli* isolation. Samples were kept in a cooler in the field and then mixed thoroughly by vortexing at the laboratory prior to freezing at −70°C (within 12 h of sample collection).

Sampling of swine feces at farms occurred between 2005 and 2008. Supplemental swine sampling occurred in 2007 to obtain samples from the farms on which the mice were trapped. For swine, each pooled sample consisted of five individual fecal samples collected from different sections of either a pen or a set of pens for a specific population (weaner, finisher, sow, etc.). Four pooled samples per farm were examined, and all samples from a farm were collected on the same date. Individual samples were pooled and homogenized before further processing. Twenty grams of each pooled fecal sample was then converted into a fecal slurry by addition of 40 ml of saline.

***E. coli* isolation.** For wild small mammals, 1.5 ml of a stored fecal suspension was centrifuged with a microcentrifuge, and the supernatant was discarded. The resulting pellet was plated on MacConkey agar (Becton Dickinson) and incubated at 37°C overnight. If lactose-fermenting colonies were obtained, four different colonies were selected at random, restreaked on nonselective media, and grown overnight at 37°C. If no lactose-fermenting colonies were obtained, 2.5 ml of the original fecal suspension was subjected to enrichment in 2.5 ml of 2× EC broth (Becton Dickinson) and grown at 37°C overnight. The next day, 100 μl of the enrichment was plated on MacConkey agar and grown overnight at 37°C. If lactose-fermenting colonies were obtained, they were subcultured on nonselective media. Presumptive identification of *E. coli* was confirmed by indole and oxidase tests. When possible, two indole-positive and oxidase-negative colonies per fecal sample were frozen at −70°C in BHI broth containing 20% glycerol for later testing. Samples which yielded no *E. coli* were not included in the study.

For swine, previously frozen fecal slurry preserved in brucella broth with 50% glycerol was used for *E. coli* isolation. One hundred microliters of slurry was plated onto MacConkey agar and incubated overnight at 37°C. Six presumptive lactose-fermenting colonies were subcultured onto secondary MacConkey agar. One colony from each secondary MacConkey agar plate was streaked on tryptic soy agar (Becton Dickinson) and incubated overnight at 37°C. Biochemical testing was conducted using indole spot reagent (PML, Mississauga, Ontario, Canada) and plating an isolated colony onto Simmons citrate agar (Becton Dickinson) to confirm identification of *E. coli*. Up to five indole-positive, citrate-negative isolates were then frozen at −86°C and used for further testing.

Isolation of *Salmonella* spp. A modified version of the established MFLP-75 *Salmonella* isolation procedure for foodstuffs was used (29). Frozen fecal samples were preenriched in buffered peptone water (Becton Dickinson) and incubated at 37°C for 24 h. One hundred microliters of an enrichment was inoculated into modified semisolid Rappaport Vassiliadis medium (Becton Dickinson). The plates were incubated at 42°C for 24 to 72 h and examined to determine whether there was a typical migration pattern. All subsequent incubations and tests were done at 37°C. Presumptive positive samples from modified semisolid Rappaport Vassiliadis medium plates were streaked onto MacConkey agar, which was followed by preparing subcultures of three non-lactose-fermenting colonies on tryptic soy agar. *Salmonella* confirmation tests were conducted using triple sugar iron, Christenssen's urea agar, and *Salmonella* O antiserum Poly A-I & Vi (Becton Dickinson).

Susceptibility tests. The antimicrobial susceptibility of all of the isolates was tested at the Laboratory for Foodborne Zoonoses (Guelph, Ontario, Canada) by using the broth microdilution method and protocols of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (12). The following antimicrobial agents were tested (breakpoints are indicated in parentheses): ampicillin (≥32 μg/ml), amoxicillin-clavulanic acid (≥32 and ≥16 μg/ml, respectively), cefoxitin (≥32 μg/ml), ceftiofur (≥8 μg/ml), ceftriaxone (≥64 μg/ml), streptomycin (≥64 μg/ml), kanamycin (≥64 μg/ml), gentamicin (≥16 μg/ml), amikacin (≥64 μg/ml), tetracycline (≥16 μg/ml), chloramphenicol (≥32 μg/ml), sulfisoxazole (≥512 μg/ml), trimethoprim-sulfamethoxazole (≥4 and ≥ 76 μg/ml, respectively), nalidixic acid (≥32 μg/ml), and ciprofloxacin (≥4 μg/ml).

Antimicrobial resistance gene detection. *E. coli* lysates were prepared as described previously (24). Briefly, bacteria were grown in 500 μl BHI broth overnight, and 20 μl of the culture was transferred to 200 μl lysis buffer (0.1 M Tris-HCl [pH 8.5], 0.05% Tween 20, 0.24 mg/ml proteinase K). The sample was incubated at 60°C for 1 h and subsequently heated at 97°C for 15 min. The β-lactamase genes bla_{TEM}, bla_{SHV}, and bla_{CMY-2} and the major genes for resis-

TABLE 2. Multiplex PCR conditions and control strains used for detection of antimicrobial resistance genes in *E. coli* isolates

PCR[a]	Gene	Primer	Primer sequence	Final primer concn (μM)	Annealing temp (°C)	Product size (bp)	Control strain
1	*sul1*	sul1-F[b]	CGGCGTGGGCTACCTGAACG	0.2	66	433	AMR 130[g]
		sul1-B[b]	GCCGATCGCGTGAAGTTCCG	0.2			
1	*sul2*	sulII-L[c]	CGGCATCGTCAACATAACCT	0.3	66	721	AMR 130[g]
		sulII-R[c]	TGTGCGGATGAAGTCAGCTC	0.3			
1	*sul3*	sul3-GKa-F[d]	CAACGGAAGTGGGCGTTGTGGA	0.2	66	244	RL0044[k]
		sul3-GKa-R[d]	GCTGCACCAATTCGCTGAACG	0.2			
2	*tet*(A)	TetA-L[c]	GGCGGTCTTCTTCATCATGC	0.1	63	502	R08[g]
		TetA-R[c]	CGGCAGGCAGAGCAAGTAGA	0.1			
2	*tet*(B)	TetBGK-F2[m]	CGCCCAGTGCTGTTGTTGTC	0.2	63	173	PB#11[g]
		TetBGK-R2[m]	CGCGTTGAGAAGCTGAGGTG	0.2			
2	*tet*(C)	TetC-L[c]	GCTGTAGGCATAGGCTTGGT	0.5	63	888	PB#2[g]
		TetC-R[c]	GCCGGAAGCGAGAAGAATCA	0.5			
3	*aadA*	4F[e]	GTGGATGGCGGCCTGAAGCC	0.1	63	525	AMR 075[g]
		4R[e]	AATGCCCAGTCGGCAGCG	0.1			
3	*strA*/*strB*	strA-F[f]	ATGGTGGACCCTAAAACTCT	0.4	63	893	AMR 075[g]
		strB-R[f]	CGTCTAGGATCGAGACAAAG	0.4			
3	*aac*(3)IV	aac4-L[g]	TGCTGGTCCACAGCTCCTTC	0.2	63	653	AMR 075[g]
		aac4-R[g]	CGGATGCAGGAAGATCAA	0.2			
4	*aadB*	aadB-L[i]	GAGGAGTTGGACTATGGATT	0.2	55	208	TN1409[h]
		aadB-R[i]	CTTCATCGGCATAGTAAAAG	0.2			
4	*aphA1*	aph(3′)-Ia F[h]	ATGGGCTCGCGATAATGTC	0.4	55	600	AMR61[g]
		aph(3′)-Ia R[h]	CTCACCGAGGCAGTTCCAT	0.4			
4	*aphA2*	aphA2-L[i]	GATTGAACAAGATGGATTGC	0.1	55	347	AMR 20[g]
		aphA2-R[i]	CCATGATGGATACTTTCTCG	0.1			
5	bla_{TEM}	GKTEMF[d]	TTAACTGGCGAACTACTTAC	0.2	55	247	TEM4676[l]
		GKTEMR[d]	GTCTATTTCGTTCATCCATA	0.2			
5	bla_{SHV}	SHV-F[j]	AGGATTGACTGCCTTTTTG	0.4	55	393	SHV4339[l]
		SHV-R[j]	ATTTGCTGATTTCGCTCG	0.4			
5	bla_{CMY-2}	CMYF[d]	GACAGCCTCTTTCTCCACA	0.2	55	1,000	R1414[d]
		CMYR[d]	TGGACACGAAGGCTACGTA	0.2			

[a] Multiplex PCR 1 were done using the following thermal cycling conditions: one cycle consisting of 15 min at 95°C, 30 cycles consisting of 1 min at 95°C, 1 min at 66°C, and 1 min at 72°C, and one cycle consisting of 10 min at 72°C. Multiplex PCR 2 and 3 were done using the following thermal cycling conditions: one cycle consisting of 15 min at 94°C, 30 cycles consisting of 1 min at 94°C, 1 min at 63°C, and 1 min at 72°C, and one cycle consisting of 10 min at 72°C. Multiplex PCR 4 and 5 were done using the following thermal cycling conditions: one cycle consisting of 15 min at 94°C, 30 cycles consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, and one cycle consisting of 10 min at 72°C.
[b] See reference 17.
[c] See reference 18.
[d] This study.
[e] See reference 20.
[f] See reference 39.
[g] See reference 3.
[h] See reference 22.
[i] See reference 41.
[j] See reference 6.
[k] See reference 27.
[l] Obtained from Mike Mulvey (Winnipeg, Manitoba).
[m] See reference 11.

tance to streptomycin (*strA*/*strB* and *aadA*), kanamycin and neomycin (*aphA1* and *aphA2*), kanamycin and gentamicin (*aadB*), apramycin, gentamicin, and tobramycin [*aac*(3)IV], sulfonamides (*sul1*, *sul2*, and *sul3*), and tetracycline [*tet*(A), *tet*(B), and *tet*(C)] were tested using a set of novel multiplex PCR protocols. The multiplex PCRs were all performed by using 25-μl mixtures and a Qiagen multiplex PCR kit (Qiagen, Mississauga, Ontario, Canada) with 1× Qiagen multiplex PCR master mixture, 1× Q-solution, and 1× primer mixture according to the manufacturer's instructions. PCR protocols and primers are described in Table 2. Three pairs of primers were used in each multiplex PCR, as shown in Table 2. The multiplex PCR for sulfonamides and aminoglycosides were each validated previously using collections of 40 isolates with known genotypes. The primers for bla_{TEM} and bla_{SHV} were designed in silico so that they

TABLE 3. Frequencies of antimicrobial resistance in *E. coli* isolates from pigs on five Ontario farms and from wild small mammals in the vicinity of these farms and in five geographically matched natural areas

Antimicrobial agent[a]	No. (%) of resistant isolates from swine (n = 125)[b]	Wild small mammals					
		Farms		Natural areas		Total	
		No. (%) of resistant isolates (n = 42)	No. (%) of animals with resistant isolates (n = 22)	No. (%) of resistant isolates (n = 37)	No. (%) of animals with resistant isolates (n = 20)	No. (%) of resistant isolates (n = 79)	No. (%) of animals with resistant isolates (n = 42)
Ampicillin	28 (22)	1 (2)	1 (5)	3 (8)	2 (15)	4 (5)	3 (7)
Amoxicillin/clavulanic acid	5 (4)	0		0		0	
Cefoxitin	3 (2)	1 (2)	1 (5)	0		1 (1)	1 (2)
Ceftiofur	3 (2)	0		0		0	
Ceftriaxone	3 (2)	0		0		0	
Streptomycin	48 (38)	3 (7)	2 (9)	0		3 (4)	2 (7)
Kanamycin	11 (9)	0		0		0	
Sulfizoxazole	62 (50)	5 (12)	3 (14)	0		5 (6)	3 (12)
Trimethoprim/sulfamethoxazole	8 (6)	1 (2)	1 (9)	0		1 (1)	1 (2)
Tetracycline	104 (83)	10 (24)	6 (27)	2 (5)	1 (5)	12 (15)	7 (29)
Chloramphenicol	13 (10)	2 (5)	1 (5)	0		2 (3)	1 (2)

[a] No resistance to amikacin, ciprofloxacin, gentamicin, and nalidixic acid was detected in *E. coli* isolates from either wild small mammals or swine.
[b] Twenty-five isolates from pooled fecal samples per farm were examined for five farms.

identified all the known variants of these genes using National Center for Biotechnology Information GenBank (Bethesda, MD) data and were validated using a smaller set of control strains kindly provided by M. Mulvey, National Microbiology Laboratory (Winnipeg, Manitoba, Canada).

Statistical analysis. For statistical analysis, all 79 *E. coli* isolates recovered from wild small mammals (pairs of isolates from 37 animals and single isolates from five animals) and 125 *E. coli* isolates obtained from swine were used. Fisher's exact tests, determination of 95% confidence intervals based on exact binomial distributions, and univariable logistic regression analysis were performed using the Stata9 statistical software (StataCorp, College Station, TX). Associations were considered significant if the P value was <0.05, and when they were significant, odds ratios (ORs) and 95% confidence intervals were calculated.

RESULTS

Recovery of *E. coli* and *Salmonella* isolates from wild small mammals. *E. coli* was isolated from 42 of 86 fecal samples (49%); however, enrichment for *E. coli* was necessary for 34 *E. coli* isolates from 18 of these samples. A total of 42 *E. coli* isolates were recovered from 22 animals trapped on farms, and 37 *E. coli* isolates were obtained from 20 animals trapped in natural areas. The proportion of animals from which *E. coli* could be recovered was not significantly higher for farms than for natural areas (P = 0.26). Details of the distribution by species and location are shown in Table 1. Despite various attempts no *Salmonella* isolates were obtained from any of the fecal samples tested (n = 49).

Prevalence of resistant *E. coli* isolates from wild small mammals. A total of 16 *E. coli* isolates (20%) from 10 animals showed reduced susceptibility to one or several antimicrobials. Thirteen resistant isolates were obtained from eight animals trapped in a farm environment, whereas three resistant isolates were obtained from two animals trapped in natural areas. The most common resistance was resistance to tetracycline, which was detected in 10 farm and 2 natural area isolates (Table 3). Resistance to ampicillin, resistance to cefoxitin, resistance to streptomycin, resistance to sulfisoxazole, resistance to trimethoprim-sulfamethoxazole, and resistance to chloramphenicol were also observed in farm isolates (Table 3). In isolates from natural areas, only resistance to ampicillin and resistance to tetracycline were detected. The frequencies of resistance in isolates from both farms and natural areas are shown in Table 3. The resistance phenotypes of isolates from the same animal were identical for five of six animals from which two *E. coli* isolates were obtained. Multiresistance profiles (resistance to two or more antimicrobial agents) were observed for isolates from three animals. These profiles included resistance to ampicillin and tetracycline (two *E. coli* isolates from one animal from a natural area) and resistance to streptomycin, sulfisoxazole, and tetracycline (two isolates from one animal from a farm); for the third animal (from a farm), one isolate showed resistance to streptomycin, sulfisoxazole, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol, while another isolate showed resistance to cefoxitin, tetracycline, and chloramphenicol.

Prevalence of resistant *E. coli* isolates from swine. Eighty-five percent of the swine isolates were resistant to one or more antimicrobials. The most common resistance was resistance to tetracycline, which was detected in 83% of the isolates (Table 3). While the most prevalent types of resistance to other antimicrobials were resistance to sulfisoxazole, resistance to streptomycin, and resistance to ampicillin, resistance to amoxicillin-clavulanic acid, resistance to cefoxitin, resistance to ceftiofur, resistance to chloramphenical, resistance to kanamycin, and resistance to trimethoprim-sulfamethoxazole were also observed. Multiresistance was observed in 73/125 isolates (58%).

Prevalence of AMR genes in wild small mammals. Altogether, AMR genes were detected in 15 *E. coli* isolates from nine animals. Thirteen of these isolates from eight animals were from farms, and two *E. coli* isolates from one animal were from a natural area. The most frequent resistance genes were *tet*(A), *tet*(B), *tet*(C), *aadA*, and *sul1* (Table 4). Some of the most frequent resistance genes found in isolates from animals from the farm environment [*aadA*, *sul1*, *tet*(B), and *tet*(C)]

TABLE 4. Frequencies of antimicrobial resistance genes in *E. coli* isolates from pigs on five Ontario farms and from wild small mammals in the vicinity of these farms and in five geographically matched natural areas

AMR gene[a]	No. (%) of isolates from swine positive for gene (n = 125)[b]	Wild small mammals					
		Farms		Natural areas		Total	
		No. (%) of isolates positive for gene (n = 42)	No. (%) of animals with one or more isolates positive for gene (n = 22)	No. (%) of isolates positive for gene (n = 37)	No. (%) of animals with one or more isolates positive for gene (n = 20)	No. (%) of isolates positive for gene (n = 79)	No. (%) of animals with one or more isolates positive for gene (n = 42)
bla_{TEM}[c]	ND[d]	ND		2 (6)	1 (5)	2 (3)	1 (2)
bla_{CMY-2}[c]	3 (2)	ND		ND		ND	
strA/*strB*	35 (28)	1 (2)	1 (5)	ND		1 (1)	1 (2)
aadA	56 (45)	5 (12)	3 (14)	ND		5 (6)	3 (7)
aphA1	10 (8)	ND		ND		ND	
aac(3)IV	3 (2)	ND		ND		ND	
sul1	22 (18)	4 (10)	2 (9)	ND		4 (5)	2 (5)
sul2	25 (20)	1 (2)	1 (5)	ND		1 (1)	1 (2)
sul3	22 (18)	ND		ND		ND	
tet(A)	33 (27)	3 (7)	2 (9)	2 (5)	1 (5)	5 (6)	3 (7)
tet(B)	72 (59)	4 (10)	3 (14)	ND		4 (5)	3 (7)
tet(C)	4 (3)	3 (7)	2 (9)	ND		3 (4)	2 (5)

[a] The resistance genes bla_{SHV}, *aadB*, and *aphA2* were not detected in either swine or wild small mammals in this study.
[b] Twenty-five isolates per farm were examined for five farms.
[c] None of the β-lactamase genes investigated (bla_{TEM}, bla_{SHV}, bla_{CMY-2}) were detected in two of the four ampicillin-resistant isolates from small wild mammals.
[d] ND, gene not detected in the isolates.

were not detected in isolates from animals trapped in natural areas. With a very few exceptions, susceptibility test results were consistent with genotyping results. Two of five isolates phenotypically susceptible to streptomycin carried genes for streptomycin resistance, and two isolates phenotypically resistant to ampicillin had none of the β-lactam resistance genes investigated. These discrepant results were confirmed by repeated testing.

There was a significant association between isolates with resistance to tetracycline and isolates collected from a farm source. The odds for tetracycline resistance were five times higher for an isolate from a farm source than for an isolate from a natural area (P = 0.02; OR, 5.00; confidence interval, 1.10 to 30.36). No specific resistance phenotype was associated with isolates from a natural area. Overall, a larger proportion of resistant *E. coli* isolates (13/42 isolates) was found in farm environments than in natural areas (3/37 isolates), but the difference was not significant (P = 0.083). No significant association between any specific resistance gene and the source of the isolates (i.e., farm versus natural area) was detected. Seven isolates carried several resistance genes simultaneously, and the following gene combinations were observed: *tet*(B) and *aadA* (two isolates from two animals); *sul1*, *aadA*, and *tet*(B) (two isolates from one animal); *tet*(A) and bla_{TEM} (two isolates from one animal); and *sul2*, *tet*(A), *aadA*, and *strA*/*strB* (one isolate from one animal). Although several resistance genes were repeatedly found together, the only significant association between AMR genes detected in wild small mammals was an association between *tet*(B) and *aadA* (P = 0.004).

Prevalence of resistance genes in swine *E. coli* isolates. Overall, AMR genes were identified in 92% of porcine *E. coli* isolates. The most common genes were, in order of decreasing prevalence, *tet*(B), *aadA*, *strA*/*strB*, and *tet*(A); other resistance genes detected are shown in Table 4. Some of the most frequent resistance genes observed in porcine *E. coli* isolates, such as *tet*(A), *tet*(B), and *aadA*, were also the genes found most frequently in isolates from wild small mammals trapped on farms. There were four discrepancies in the tetracycline phenotypes and genotypes of swine isolates. Three isolates contained *tet*(B) but were phenotypically susceptible, and one isolate contained *tet*(*C*) but was classified as susceptible. Twenty-eight isolates classified as streptomycin susceptible carried a resistance gene (21 isolates carried *aadA*, 5 isolates carried *strA*/*strB*, and 2 isolates carried both *aadA* and *strA*/*strB*). Twenty-eight isolates were phenotypically resistant to ampicillin, but only three isolates carried one of the resistance genes investigated. The strongest significant associations between resistance genes in swine isolates were the associations between *sul1* and *aadA* (OR, 18.33), between *sul2* and *aphA1* (OR, 23.06), between *tet*(A) and *tet*(B) (OR, 0.008), and between *aphA1* and *strA*/*strB* (OR, 13.04). The remaining associations between resistance genes and their corresponding ORs and confidence intervals are shown in Table 5. The only association observed in the wild small mammals [*tet*(B) and *aadA*] was also found in the swine isolates, although it was not the most prominent association.

DISCUSSION

Compared to the rate of isolation of *E. coli* isolates from swine samples, the rate of isolation of *E. coli* isolates from wild small mammal samples was low. Loss of *E. coli* viability caused by freezing of the fecal samples cannot be excluded as a reason for this low recovery rate. However, storage in the presence of 10% glycerol as a cryoprotectant is usually considered a safe way to store fecal samples at low temperatures for later recov-

TABLE 5. Statistically significant associations between AMR genes in 125 *E. coli* isolates from pigs on five Ontario farms

Gene association[a]	*P* value	OR	95% confidence interval
sul1 + *aac*(3)IV	0.005	—[b]	—
su11 + *aadA*	≤0.001	18.33	3.43–97.94
sul2 + *sul3*	0.007[c]		
sul2 + *aphA1*	≤0.001	23.06	3.70–143.45
sul2 + *strA*/*strB*	≤0.001	9.68	3.21–29.15
sul3 + *tet*(A)	≤0.001	6.15	2.13–17.72
sul3 + bla_{CMY-2}	0.005	—	—
sul3 + *aac*(3)IV	0.005	—	—
tet(A) + *tet*(B)	≤0.001	0.008	0.00–0.14
tet(A) + *strA*/*strB*	0.015	0.26	0.08–0.84
tet(B) + *tet*(C)	0.026[c]	—	—
tet(B) + *strA*/*strB*	≤0.001	19.20	3.62–101.809
tet(B)+ *aadA*	0.008	2.75	1.25–6.06
aphA1 + *strA*/*strB*	≤0.001	13.037	2.34–72.7

[a] Only significant associations ($P < 0.05$) between genes are shown.
[b] —, despite the presence of a significant association, the OR and the confidence interval could not be estimated reliably because of the presence of a zero in one or several of the cells in the two-by-two table used for testing pairwise associations.
[c] Negative association (i.e., incompatible genes).

ery of nonfastidious organisms such as *Enterobacteriaceae*. Other studies have demonstrated that the use of glycerol as a cryoprotectant allows high rates of recovery of *E. coli*, close to the rates obtained with fresh samples (40). Sayah et al. isolated *E. coli* from between 9 and 61% of fecal samples collected from a variety of different species (34), suggesting that the rate of *E. coli* recovery may be different for different species. Although *E. coli* isolation rates were typically not reported in previous studies of AMR in wild small mammals (10, 26), results similar to our results were obtained in a Polish study in which *E. coli* isolates were isolated from only 20% of wild voles (38). Recovery of *E. coli* from laboratory mice has also been shown to be problematic (16).

Despite various attempts, no *Salmonella* isolate was detected in any of the fecal samples tested. These results support the finding of other researchers that *Salmonella* occurs very infrequently in wild small animals (13, 15, 28). The low rate of occurrence of *E. coli* and the absence of *Salmonella* in the gastrointestinal tracts of wild small mammals may be attributable to the diet of these animals, which can vary depending on the geographic location, the population density, and seasonal variations in the food supply (21, 38). The resulting requirement for enrichment to recover *E. coli* may have allowed some strains to overgrow other strains during the process, potentially resulting in decreased diversity. This may have biased our results and explain why the majority of *E. coli* isolates from the same animal had identical resistance patterns.

Tetracycline resistance was by far the most common type of resistance observed in the wild small-mammal isolates and was significantly associated with farm origin. This is not surprising since tetracycline is often used as a first-line antimicrobial in disease prevention and growth promotion in food animals, and its widespread use has likely contributed to high rates of resistance (30). The frequency of tetracycline resistance in the pigs from the farms that we investigated was 83%, which is within the range of values described in previous reports (68 to 93%) (3, 19, 22, 37). Since tetracycline resistance genes are located on mobile genetic elements, they are transmissible between bacteria (31), and it is likely that either the wild small mammals exposed to bacteria from swine or other farm sources were colonized by these bacteria or their resident flora acquired tetracycline resistance determinants from these bacteria through horizontal gene transfer. Since AMR can be selected by antimicrobials in feed (8), it is also possible that *E. coli* isolates from some wild small mammals were directly exposed to selection pressure through animal feed containing antimicrobials, such as tetracycline.

As observed on the farms investigated here, resistance to sulfonamides and streptomycin occurs frequently in bacteria from swine (3, 12, 18). Despite a lack of a significant association with farm origin, it was nevertheless not surprising to detect resistance to these antimicrobials in the small mammals trapped in the vicinity of farms. The rate of resistance to streptomycin was within the range reported in other studies of wild animals (0 to 7%) (26, 32, 34); however, resistance to sulfonamides in wild small mammals has not been reported previously by other workers (26, 34). This difference between studies may reflect differences in antimicrobial use in swine and other livestock between countries.

Resistance to amoxicillin-clavulanic acid does not occur frequently in *E. coli* isolates from farm animals in Canada (12), and only 2.4% of our swine isolates were resistant to this antibiotic-inhibitor combination. None of the *E. coli* isolates from small mammals examined was resistant to amoxicillin-clavulanic acid. This is in strong contrast to the results of a British study which found that 97% of *E. coli* isolates from similar animal species (bank voles and wood mice) were resistant to amoxicillin-clavulanate (10). However, our results are consistent with the results of another study (26), which did not detect resistance of this type. The high resistance rate observed by Gilliver and collaborators may have been due to higher human population density in the study area (26). Alternatively, the fact that ampicillin resistance was the only type of resistance observed besides tetracycline resistance and occurred most frequently in isolates from wild small mammals from natural areas in this study may agree with the results of Gilliver and coworkers, suggesting that there may be a natural source of selection for resistance to β-lactams in these animals. Finally, despite the absence of a significant association with the origin of the small mammals, the presence of resistance to chloramphenicol in two *E. coli* isolates from one wild mammal (*Peromyscus* sp.) from a farm environment and in approximately 10% of our swine isolates suggests that there may be direct or indirect transmission from farm animals to wildlife. Chloramphenicol was banned over 20 years ago in Canada, but the genes are known to persist in porcine *E. coli* isolates (41).

With a few exceptions, the correlation between antimicrobial resistance and the presence of AMR genes was good. For streptomycin, a discrepancy between genotype and phenotype was expected, because previous studies have shown that streptomycin resistance genes can be detected in isolates classified as susceptible, suggesting that the breakpoint used for this antimicrobial may be too high for epidemiological purposes (3, 18). The apparent absence of β-lactam resistance genes in ampicillin-resistant isolates strongly suggests that the observed resistance was caused either by β-lactamases other than those investigated here but identified in other studies (33) or by

other resistance mechanisms, such as efflux pumps or changes in porins and other cell wall components (1).

The relatively small number of resistant isolates recovered from wild small mammals did not allow detailed numerical estimates of the distribution of AMR genes to be obtained and limited the power of statistical investigations. However, despite the lack of significant differences between the gene distribution in small wild mammals from farms and the gene distribution in small wild mammals from natural areas, the majority of genes found in *E. coli* isolates from the animals captured around swine farms were also among the most frequent genes in isolates from swine. For instance, the integron-associated genes *aadA* and *sul1* (9) were among the most frequent AMR genes in *E. coli* isolates from wild small mammals from farm environments and swine but were not found in wild small mammals from natural areas. All three tetracycline resistance genes investigated were found in isolates from both swine and wild small mammals from the farm environment. Like the integron-associated genes, *tet*(B) was also a predominant gene in isolates from both of these sources, but it was not detected in the few tetracycline-resistant isolates from wild mammals in natural areas. This AMR gene has also been found in other wildlife studies (7) and is the most common tetracycline resistance gene in generic *E. coli* isolates from domestic and farm animals in general (4, 18).

Almost one-half of all resistant isolates from wild small mammals showed resistance to several antimicrobial agents and carried more than one resistance gene. Because of the relatively small sample size, a statistically significant association between genes was detected only for *tet*(B) and *aadA* in these isolates. Probably because of the much higher overall prevalence of resistance, numerous associations between AMR genes were detected in the isolates from swine. Such statistical associations are usually the result of AMR gene linkage on single mobile genetic elements rather than a result of independent acquisition of multiple resistance genes. The only significant pairwise gene association observed in wild small mammals was an association of genes detected in porcine isolates. Due to the small sample size it is difficult to assess the significance of this finding, but the same common mobile genetic element carrying *tet*(B) and *aadA* may have spread between the two populations.

In conclusion, this study demonstrated that AMR determinants were present in wild small mammals from both natural areas and swine farms. Although the overall resistance rate was low and a statistically significant association between the source of the wild small mammals and resistance was demonstrated only for tetracycline, the observed frequencies of AMR and associated genetic determinants suggest that wild mammals living in the proximity of farms are generally more likely to harbor resistant bacteria than wild mammals living in natural areas. There were commonalities between the molecular patterns of the isolates from wild small mammals and the molecular patterns of the isolates from pigs on the swine farms investigated, but a larger sample size is needed to statistically test this apparent association. Thus, the results of this study suggest that agricultural activities, specifically antimicrobial use (in this case in swine farming), may have a significant impact on AMR observed in nature. More studies with larger samples and more precise AMR gene typing by DNA sequencing and molecular typing of bacterial strains are needed before further hypotheses concerning the exact routes of transmission and what may drive the resistance rates can be precisely formulated.

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REFERENCES

1. **Amyes, S. G.** 2003. Resistance to beta-lactams—the permutations. J. Chemother. **15:**525–535.
2. **Blanco, G., J. A. Lemus, J. Grande, L. Gangoso, J. M. Grande, J. A. Donazar, B. Arroyo, O. Frias, and F. Hiraldo.** 2007. Geographical variation in cloacal microflora and bacterial antibiotic resistance in a threatened avian scavenger in relation to diet and livestock farming practices. Environ. Microbiol. **9:**1738–1749.
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. **Bryan, A., N. Shapir, and M. J. Sadowsky.** 2004. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical *Escherichia coli* strains isolated from diverse human and animal sources. Appl. Environ. Microbiol. **70:**2503–2507.
5. **Cole, D., D. J. Drum, D. E. Stalknecht, D. G. White, M. D. Lee, S. Ayers, M. Sobsey, and J. J. Maurer.** 2005. Free-living Canada geese and antimicrobial resistance. Emerg. Infect. Dis. **11:**935–938.
6. **Colom, K., J. Perez, R. Alonso, A. Fernandez-Aranguiz, E. Larino, and R. Cisterna.** 2003. Simple and reliable multiplex PCR assay for detection of *bla*TEM, *bla*(SHV) and *bla*OXA-1 genes in *Enterobacteriaceae*. FEMS Microbiol. Lett. **223:**147–151.
7. **Costa, D., P. Poeta, Y. Saenz, L. Vinue, B. Rojo-Bezares, A. Jouini, M. Zarazaga, J. Rodrigues, and C. Torres.** 2006. Detection of *Escherichia coli* harbouring extended-spectrum beta-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. J. Antimicrob. Chemother. **58:**1311–1312.
8. **Diarra, M. S., F. G. Silversides, F. Diarrassouba, J. Pritchard, L. Masson, R. Brousseau, C. Bonnet, P. Delaquis, S. Bach, B. J. Skura, and E. Topp.** 2007. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and enterococcus number, antibiotic resistant phenotype, and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Appl. Environ. Microbiol. **73:**6566–6576.
9. **Fluit, A. C., and F. J. Schmitz.** 2004. Resistance integrons and super-integrons. Clin. Microbiol. Infect. **10:**272–288.
10. **Gilliver, M. A., M. Bennett, M. Begon, S. M. Hazel, and C. A. Hart.** 1999. Antibiotic resistance found in wild rodents. Nature **401:**233–234.
11. **Goswami, P. S., C. L. Gyles, R. M. Friendship, C. Poppe, G. K. Kozak, and P. Boerlin.** 2008. Effect of plasmid pTENT2 on severity of porcine postweaning diarrhoea induced by an O149 enterotoxigenic *Escherichia coli*. Vet. Microbiol. **131:**400–405.
12. **Government of Canada.** 2007. Canadian integrated program for antimicrobial resistance surveillance (CIPARS) 2005. Public Health Agency of Canada, Guelph, Ontario.
13. **Healing, T. D., C. Kaplan, and A. Prior.** 1980. A note on some Enterobacteriaceae from faeces of small wild British mammals. J. Hyg. **85:**343–345.
14. **Hudson, C. R., C. Quist, M. D. Lee, K. Keyes, S. V. Dodson, C. Morales, S. Sanchez, D. G. White, and J. J. Maurer.** 2000. Genetic relatedness of *Salmonella* isolates from nondomestic birds in southeastern United States. J. Clin. Microbiol. **38:**1860–1865.
15. **Jones, P. W., and G. I. Twigg.** 1976. Salmonellosis in wild mammals. J. Hyg. **77:**51–54.
16. **Kasman, L.** 2005. Barriers to coliphage infection of commensal intestinal flora of laboratory mice. Virol. J. **2:**34–40.
17. **Kerrn, M. B., T. Klemmensen, N. Frimodt-Moller, and F. Espersen.** 2002. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. J. Antimicrob. Chemother. **50:**513–516.
18. **Lanz, R., P. Kuhnert, and P. Boerlin.** 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. **91:**73–84.

19. **Lee, C., B. E. Langlois, and K. A. Dawson.** 1993. Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial agent exposure. Appl. Environ. Microbiol. **59:**1467–1472.
20. **Madsen, L., F. M. Aarestrup, and J. E. Olsen.** 2000. Characterization of streptomycin resistance determinants in Danish isolates of *Salmonella typhimurium*. Vet. Microbiol. **75:**73–82.
21. **Maron, J. L., and M. J. Kauffman.** 2006. Habitat-specific impacts of multiple consumers on plant population dynamics. Ecology **87:**113–124.
22. **Maynard, C., J. M. Fairbrother, S. Bekal, F. Sanschagrin, R. C. Levesque, R. Brousseau, L. Masson, S. Lariviere, and J. Harel.** 2003. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Chemother. **47:**3214–3221.
23. **Mills, J. N., T. L. Yates, J. E. Childs, R. R. Parmenter, T. G. Ksiazek, and P. E. Rollin.** 1995. Guidelines for working with rodents potentially infected with hantavirus. J. Mammal. **76:**716–722.
24. **Miserez, R., J. Frey, C. Buogo, S. Capaul, A. Tontis, A. Burnens, and J. Nicolet.** 1998. Detection of alpha- and epsilon-toxigenic *Clostridium perfringens* type D in sheep and goats using a DNA amplification technique (PCR). Lett. Appl. Microbiol. **26:**382–3866.
25. **O'Rourke, K.** 2003. Antimicrobial resistance in wildlife: it's making a bigger splash than you think. J. Am. Vet. Med. Assoc. **223:**756–757.
26. **Osterblad, M., K. Norrdahl, E. Korpimaki, and P. Huovinen.** 2001. Antibiotic resistance. How wild are wild mammals? Nature **409:**37–38.
27. **Perreten, V., and P. Boerlin.** 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob. Agents Chemother. **47:**1169–1172.
28. **Pocock, M. J. O., J. B. Searle, W. B. Betts, and P. C. L. White.** 2001. Patterns of infection by *Salmonella* and *Yersinia* spp. in commensal house mouse (*Mus musculus domesticus*) populations. J. Appl. Microbiol. **90:**755–760.
29. **Poppe, C., E. D. Mann, S. Shaw, D. Warburton, and A. Sewell.** 2004. Procedure for the isolation of *Salmonella* species by the modified semi-solid Rappaport Vassilliadis (MSRV) method. Government of Canada, Guelph, Ontario http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/res-rech/mflp75-eng.pdf.
30. **Roberts, M. C.** 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiol. Rev. **19:**1–24.
31. **Roberts, M. C.** 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol. Lett. **245:**195–203.
32. **Routman, E., R. D. Miller, J. Phillips-Conroy, and D. L. Hartl.** 1985. Antibiotic resistance and population structure in *Escherichia coli* from free-ranging African yellow baboons. Appl. Environ. Microbiol. **50:**749–754.
33. **Samaha-Kfoury, J. N., and G. F. Araj.** 2003. Recent developments in beta lactamases and extended spectrum beta lactamases. BMJ **327:**1209–1213.
34. **Sayah, R. S., J. B. Kaneene, Y. Johnson, and R. Miller.** 2005. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. Appl. Environ. Microbiol. **71:**1394–1404.
35. **Skurnik, D., R. Ruimy, A. Andremont, C. Amorin, P. Rouquet, B. Picard, and E. Denamur.** 2006. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. J. Antimicrob. Chemother. **57:**1215–1219.
36. **Souza, V., M. Rocha, A. Valera, and L. E. Eguiarte.** 1999. Genetic structure of natural populations of *Escherichia coli* in wild hosts on different continents. Appl. Environ. Microbiol. **65:**3373–3385.
37. **Stine, O. C., J. A. Johnson, A. Keefer-Norris, K. L. Perry, J. Tigno, S. Qaiyumi, M. S. Stine, and J. G. Morris, Jr.** 2007. Widespread distribution of tetracycline resistance genes in a confined animal feeding facility. Int. J. Antimicrob. Agents **29:**348–352.
38. **Swiecicka, I., J. Buczek, and A. Iwaniuk.** 2003. Analysis of genetic relationships and antimicrobial susceptibility of *Escherichia coli* isolated from *Clethrionomys glareolus*. J. Gen. Appl. Microbiol. **49:**315–320.
39. **Tamang, M. D., J. Y. Oh, S. Y. Seol, H. Y. Kang, J. C. Lee, Y. C. Lee, D. T. Cho, and J. Kim.** 2007. Emergence of multidrug-resistant *Salmonella enterica* serovar Typhi associated with a class 1 integron carrying the dfrA7 gene cassette in Nepal. Int. J. Antimicrob. Agents **30:**330–335.
40. **Ternent, H. E., G. T. Innocent, L. M. Filshie, D. J. Taylor, W. B. Steele, S. A. McEwen, W. J. Reilly, G. J. Gunn, S. W. Reid, and D. J. Mellor.** 2004. Frozen storage of *Escherichia coli* O157 in buffered peptone water and its detection on bovine carcasses. J. Food Prot. **67:**40–45.
41. **Travis, R. M., C. L. Gyles, R. Reid-Smith, C. Poppe, S. A. McEwen, R. Friendship, N. Janecko, and P. Boerlin.** 2006. Chloramphenicol and kanamycin resistance among porcine *Escherichia coli* in Ontario. J. Antimicrob. Chemother. **58:**173–177.