Pilot Study of Antimicrobial Resistance in Northern Bobwhites (*Colinus virginianus*)

Author(s): Michael Zhang, Zhenyu Shen, Dale Rollins, William Fales, and Shuping Zhang
Source: Avian Diseases, 61(3):391-396.
Published By: American Association of Avian Pathologists
https://doi.org/10.1637/11629-031517-RegR
URL: http://www.bioone.org/doi/full/10.1637/11629-031517-RegR

AVIAN DISEASES 61:391–396, 2017

Research Note—

Pilot Study of Antimicrobial Resistance in Northern Bobwhites (*Colinus virginianus*)

Michael Zhang,[A] Zhenyu Shen,[B] Dale Rollins,[C] William Fales,[B] and Shuping Zhang[BD]

[A]Department of Biomedical Sciences and Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211
[B]Department of Veterinary Pathobiology and Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211
[C]Rolling Plains Quail Research Foundation, Roby, TX 76956

Received 17 March 2017; Accepted 6 June 2017; Published ahead of print 3 July 2017

SUMMARY. Antimicrobial resistance (AMR) is an important issue for both wildlife conservation and public health. The purpose of this study was to screen for AMR in fecal bacteria isolated from northern bobwhite (*Colinus virginianus*), a species that is an ecologically and economically important natural resource in the southern United States. The antimicrobial susceptibility profiles of 45 *Escherichia coli* isolates, 20 *Enterococcus faecalis* isolates, and 10 *Enterococcus faecium* isolates were determined using the Sensititer™ microbroth dilution minimum inhibitory concentration (MIC) plate, AVIAN1F. Overall, *E. coli* isolates had high MIC values for the following classes of antimicrobials: aminocoumarins, beta-lactams, lincosamides, macrolides, florfenicol, and sulfonamides. *Enterococcus faecalis* and *E. faecium* isolates had high MICs for aminocyclitols, aminoglycosides, beta-lactams, lincosamides, and sulfonamides. *Enterococcus faecalis* isolates also showed high MICs for aminocoumarins, while *E. faecium* isolates had high MICs for trimethoprim/sulfamethoxazole and tetracycline. Based on available veterinary interpretive criteria, 15% and 33% of *E. coli* isolates were resistant to sulphathiazole and sulphadimethoxine, respectively. Intermediate susceptibility to florfenicol was seen with 17.8% of *E. coli* isolates. Twenty percent of *E. faecalis* and 80% of *E. faecium* isolates were resistant to high-concentration streptomycin. One third of *E. faecalis* and 70% of *E. faecium* isolates were intermediately susceptible to erythromycin. Ten percent of *E. faecium* isolates were resistant to tetracycline and oxytetracycline. A comparison of available MIC suggests that AMR in wild bobwhite is less severe than in domestic poultry. Further investigation is needed to determine the source of AMR in wild bobwhite.

RESUMEN. *Nota de investigación*- Estudio piloto de la resistencia a los antimicrobianos en codornices cotuís (*Colinus virginianus*) del Norte.

La resistencia antimicrobiana (AMR) es un tema importante tanto para la conservación de la vida silvestre como para la salud pública. El propósito de este estudio fue la determinación de la resistencia antimicrobiana en bacterias fecales aisladas de codornices cotuís (*Colinus virginianus*) del Norte, una especie que es un recurso natural ecológicamente y económicamente importante en el sur de los Estados Unidos. Se determinaron los perfiles de susceptibilidad antimicrobiana de 45 aislados de *Escherichia coli*, de 20 aislados de *Enterococcus faecalis* y de 10 aislados de *Enterococcus faecium* utilizando placas Sensititer™ con código AVIAN1F, para determinar la concentración mínima inhibitoria (MIC) mediante dilución en cultivo. En general, los aislamientos de *E. coli* presentaron valores de concentraciones mínimas inhibitorias altos para las siguientes clases de antimicrobianos: aminocumarinas, beta-lactamicos, lincosamidas, macrólidos, florfenicol y sulfonamidas. Los aislados de *E. faecalis* y *E. faecium* mostraron valores altos de concentraciones mínimas inhibitorias para los aminociclitoles, aminoglucósidos, beta-lactamicos, lincosamidas y sulfonamidas. Los aislados de *Enterococcus faecalis* también mostraron valores altos de concentraciones mínimas inhibitorias para las aminocumarinas, mientras que los aislados de *E. faecium* tuvieron altas concentraciones mínimas inhibitorias para la trimetoprima/sulfametoxazol y la tetraciclina. Basándose en los criterios de interpretación veterinaria disponibles, el 15% y el 33% de los aislamientos de *E. coli* fueron resistentes al sulfatiazol y a la sulfadimetoxina, respectivamente. Se observó susceptibilidad intermedia para el florfenicol con 17.8% de los aislamientos de *E. coli*. El 20% de los aislamientos de *E. faecalis* y el 80% de los aislamientos de *E. faecium* aislados fueron resistentes a la estreptomicina de alta concentración. Un tercio de los aislados de *E. faecalis* y el 70% de los aislamientos de *E. faecium* aislados mostraron una susceptibilidad intermedia a la eritromicina. El 10% de los aislamientos de *E. faecium* fueron resistentes a la tetraciclina y a la oxitetraciclina. Una comparación de las concentraciones mínimas inhibitorias disponibles sugiere que la resistencia antimicrobiana en las codornices cotuís silvestres es menos severa que en las aves domésticas. Se necesitan más investigaciones para determinar la fuente de resistencia antimicrobiana en las especie codornices cotuís silvestres.

Key words: antimicrobial resistance, bacteria, *Colinus virginianus*, minimum inhibitory concentration, Northern Bobwhite

Abbreviations: AMOX = amoxicillin; AMR = antimicrobial resistance; CLI = clindamycin; CLSI = Clinical Laboratory Standards Institute; ENRO = enrofloxacin; ERY = erythromycin; FFN = florfenicol; GEN = gentamicin; MIC = minimum inhibitory concentration; NARMS = National Antimicrobial Resistance Monitoring System for Enteric Bacteria; NEO = neomycin; NOV = novobiocin; OXY = oxytetracycline; PEN = penicillin; SDM = sulphadimethoxine; SPE = spectinomycin; STR = streptomycin; STZ = sulphathiazole; SXT = trimethoprim/sulfamethoxazole; TET = tetracycline; TYLT = tylosin tartrate; XNL = ceftiofur

The northern bobwhite (*Colinus virginianus*) is an ecologically and economically important natural resource in the southern United States (30). Bobwhite populations have been declining since the late 1970s, which threatens the extinction of this species and the long-

[D]Corresponding author. E-mail: zhangshup@missouri.edu

treasured tradition of quail hunting, especially in southern states of the United States (28). Some private farms and plantations release pen-reared bobwhite birds to the wild in an attempt to increase the breeding populations of wild birds. One study shows about 40% of released birds can survive the initially heavy mortality and about 25% can survive to the end of the hunting season (9). This raises the question of whether pen-reared birds share their intestinal microbes, some of which could be resistant to antimicrobial agents commonly used in avian medicine. Another concern is whether human activities introduce antimicrobial resistance (AMR) to the habitats of wild bobwhite populations or bobwhite would serve as an AMR reservoir or transmission vehicle.

Antimicrobial resistance is an increasingly serious threat to global public health and animal health (4,31). Each year in the United States, more than 2 million people become infected with AMR bacteria, and about 23,000 deaths occur as a direct result of these infections (5). It is believed that the transfer of resistant bacteria and resistance genes from animal to humans contribute to the rapid emergence of AMR in human health care (15,29). Since the first report of antimicrobial resistant bacteria originating from wild birds (24), AMR has been detected in various bird species, including waterfowl, predatory, and scavenger birds and even wild birds in the Arctic (16,17,19,25,26). The present study was undertaken as part of a larger project named "Operation Idiopathic Decline," which aimed to understand the various health issues of wild bobwhite populations in the Rolling Plains ecoregion of Texas and Oklahoma, USA (27). The purpose of the present pilot study was to determine the prevalence and profile of AMR in fecal indicator bacteria isolated from the intestinal contents of wild-caught bobwhite birds from western Texas and western Oklahoma. The data will be used to direct future study on the health and management of wild bobwhite populations and impact of releasing pen-reared birds on the health of wild populations.

MATERIALS AND METHODS

Ethical consideration. Animal and tissue uses were approved by the Institutional Animal Care and Use Committees of Texas A&M University (IACUC 2011-193) and Texas Tech University (IACUC 11049-07). Trapping practices were conducted under the auspices of a Texas Parks & Wildlife Department Scientific Collector's permit.

Sample collection and bacterial culture. Sample collection and processing were completed by the Central Specimen Receiving, Processing, and Distribution Laboratory of the Institute of Environmental and Human Health, Texas Tech University. For the original "Operation Idiopathic Decline" project, a total of 2,615 birds on 33 private ranches and state-owned wildlife management areas were trapped across the 3 yr of the study. About 590 birds were subjected to microbiology study. The sampled area was approximately 9 million hectares in the Rolling Plains ecoregion of Texas and Oklahoma. The birds were euthanatized, and tissue samples were utilized for various laboratory testing. All samples were stored at −20°C prior to culture. The contents of ceca and large intestine were sampled with sterile polyester swabs (Puritan Medical, Guilford, ME, USA) and inoculated onto Tryptic Soy Agar with 5% Sheep Blood and Columbia Colistin Nalidixic Acid agar with 5% Sheep Blood and MacConkey agar, and in Brain Heart Infusion broth. Inoculated Tryptic Soy Agar and Columbia Colistin Nalidixic Acid agar plates were incubated at 37°C in either 5% CO_2, whereas MacConkey agar plates and Brain Heart Infusion broth were incubated in ambient air followed by daily inspection for 5 days. After an overnight incubation, broth was inoculated onto agar plates followed by a 5-day incubation. Presumptive *Escherichia coli* and *Enterococcus* spp. colonies were selected and purified for further identification and susceptibility testing. Bacterial identification was conducted using the MALDI Biotyper and supplementary biochemical characterizations, including oxidase, catalase, urease, and indole test. One *E. coli* and one *Enterococcus* isolate per bird were included in the subsequent antimicrobial susceptibility study.

Antimicrobial susceptibility testing. The antimicrobial susceptibility or resistance profile was determined using the Sensititer^{TM} microbroth dilution minimum inhibitory concentration panel, namely, AVIAN1F (Trek Diagnostic Systems Inc., Westlake, OH). The antimicrobial susceptibility plate included 18 antibiotics representing 10 different classes: amoxicillin, ceftiofur, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, neomycin, novobiocin, oxytetracycline, penicillin, spectinomycin, streptomycin, sulphadimethoxine, sulphathiazole, tetracycline, trimethoprim-sulfamethoxazole, and tylosin tartrate. Susceptibility assays were carried out according to Clinical Laboratory Standards Institute (CLSI) guidelines for broth microdilution methods (7,8). *E. coli* ATTC 25922 and *Enterococcus faecalis* ATCC 29212 were used as the quality control strains. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antimicrobial agent that inhibited visible bacterial growth. MIC_{50} and MIC_{90} were determined as the minimum concentrations of an antimicrobial agent at which the growth of 50% and 90% of isolates were inhibited, respectively. Veterinary-specific interpretive criteria were obtained from CLSI and Antimicrobial Therapy in Veterinary Medicine. Interpretive criteria for high-level streptomycin was obtained from the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) (6,11).

RESULTS

A total of 45 *E. coli*, 20 *E. faecalis,* and 10 *Enterococcus faecium* isolates were recovered from 155 quail intestinal contents. The MIC distribution and susceptibility patterns of *E. coli* isolates are shown in Table 1 and Table 2, respectively. Overall, *E. coli* isolates had high MIC values for the following classes of antimicrobials: aminocoumarins, beta-lactams, lincosamides, macrolides, florfenicol, and sulfonamides. Based on available veterinary interpretive criteria, all *E. coli* isolates (100%) were resistant to novobiocin. About 15% and 33% of *E. coli* isolates were resistant to sulphathiazole and sulphadimethoxine, respectively. Intermediate susceptibility to florfenicol was seen with 17.8% of isolates. All *E. coli* isolates were susceptible to spectinomycin, aminoglycosides, ceftiofur, enrofloxacin, tetracycline, and trimethoprim/ sulfamethoxazole.

The MIC distribution and susceptibility pattern of *E. faecalis* and *E. faecium* are shown in Table 3, Table 4, and Table 5, respectively. *Enterococcus faecalis* and *E. faecium* isolates had high MICs for the following classes of antimicrobials: aminocyclitols, aminoglycosides, beta-lactams, lincosamides, and sulfonamides. *Enterococcus faecalis* isolates also showed high MICs for aminocoumarins while *E. faecium* isolates had high MICs for trimethoprim/sulfamethoxazole and tetracycline. Using available veterinary interpretive criteria, 45% of *E. faecalis* isolates were resistant to novobiocin. Twenty percent of *E. faecalis* and 80% of *E. faecium* isolates were resistant to high-concentration streptomycin, respectively. One third of *E. faecalis* and 70% of *E. faecium* isolates were intermediately susceptible to erythromycin. Ten percent of *E. faecium* isolates were resistant to tetracycline and oxytetracycline. All *Enterococcus* isolates were susceptible to penicillin and tylosin tartrate. All *E. faecalis* isolates were also susceptible to tetracycline, and all *E. faecium* isolates were susceptible to novobiocin.

Table 1. MIC (μg/ml) distribution (%) of *E. coli* isolates ($n = 45$).

Class	Agents*	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	MIC50	MIC90
Aminocoumarins	NOV				0	0	0	**100**									**>4**	**>4**
Aminocyclitols	SPE								40	60	0	0					≤8	16
Aminoglycosides	GEN				73	12	0	0	0								≤0.5	1
	NEO						100	0	0	0	0						≤2	≤2
	STR								100	0	0	0	0	0	0	0	≤8	≤8
Beta-lactams	AMOX			0	0	0	38	49	11	2							4	8
	XNL			53	47	0	0	0									0.25	0.5
	PEN	0	0	0	0	0	0	0	**100**								**>8**	**>8**
Florfenicols	FFN					7	31	51	11								4	8
Fluoroquinolones	ENRO		100	0	0	0	0										≤0.12	≤0.12
Lincosamides	CLI				0	0	0	**100**									>4	>4
Sulfonamides	SDM										16	16	27	**42**			128	**>256**
	STZ										69	7	9	**16**			≤32	**>256**
	SXT				100	0	0										≤0.5	≤0.5
Tetracyclines	OXY			0	9	71	20	0	0								1	9
	TET				18	53	29										1	2
Macrolides	ERY		0	0	0	0	0	**100**									**>4**	**>4**
	Agent						2.5	5	10	20							MIC50	MIC90
	TYLT						0	0	0	**100**							**>20**	**>20**

*Antimicrobial agents: SPE, spectinomycin; NOV, novobiocin; GEN, gentamicin; NEO, neomycin; STR, streptomycin; AMOX, amoxicillin; XNL, ceftiofur; PEN, penicillin; ENRO, enrofloxacin; CLI, clindamycin; FFN, florfenicol; SDM, sulphadimethoxine; STZ, sulphathiazole; SXT, trimethoprim/sulfamethoxazole; OXY, oxytetracycline; TET, tetracycline; ERY, erythromycin; and TYLT, tylosin tartrate. The shaded areas indicate the concentrations not tested. Bold numbers indicate that isolates had MIC values greater than the highest concentration tested.

DISCUSSION

Although AMR occurs naturally through genetic mutation and acquisition of resistance genes from other bacteria, misuse of antimicrobials, and poor biosecurity measures can accelerate the development and spread of AMR (31). Wild birds have been tested as sentinels for the detection of AMR and zoonotic pathogens because of their direct contacts with humans, animals, and the environment (20,21). On the other hand, concerns have been raised over the role of wild birds in maintaining and spreading AMR and pathogens (17,25,26). To understand the epidemiology of AMR in bobwhite populations, we have determined the AMR patterns of *E. coli* and *Enterococcus* spp. isolated from bobwhite intestinal contents. These bacterial species are commensal organisms that are excreted into the environment and occasionally cause infections in domestic and wild animals as well as humans. Due to their wide distribution, *E. coli* and *Enterococci* are frequently used as biological indicators of fecal pollution in the water and the environment (20,23). In the United States, the prevalence of AMR *E. coli* and *Enterococci* in retail beef, chicken, and turkey is routinely monitored by NARMS. In the present study, we utilized a commercial antimicrobial susceptibility

Table 2. Antimicrobial susceptibility pattern of *E. coli* isolates based on MIC breakpoints for veterinary pathogens.

		Breakpoints				% Isolates		
Class	Antimicrobial agents*	S	I	R	Reference**	S	I	R
Aminocoumarins	NOV	≤4	8	>16	#2	0.0	0.0	**100.0**
Aminocyclitols	SPE	≤20	—	—	#2	100.0	0.0	0.0
Aminoglycosides	GEN	≤2	4	≥8	#1	100.0	0.0	0.0
	NEO	≤8	16	>32	#2	100.0	0.0	0.0
	STR	≤32	—	≥64	#3	100.0	—	0.0
Beta-lactams	XNL	≤2	4	≥8	#3	100.0	0.0	0.0
Florfenicols	FFN	≤4	8	≥16	#1	82.2	**17.8**	0.0
Fluoroquinolones	ENRO	≤0.25	0.5–1	≥2	#1	100.0	0.0	0.0
Sulfonamides	SDM	≤256	—	≥512	#1	66.7	—	**33.3**
	STZ	≤256	—	≥512	#1	84.4	—	**15.6**
	SXT	≤2/38	—	≥4/76	#1	100.0	—	0.0
Tetracyclines	OXY	≤4	8	≥16	#1	100.0	0.0	0.0
	TET	≤4	8	≥16	#1	100.0	0.0	0.0

*Antimicrobial agents: NOV, novobiocin; SDM, sulphadimethoxine; STZ, sulphathiazole; FFN, florfenicol; SPE, spectinomycin; GEN, gentamicin; NEO, neomycin; STR, streptomycin; XNL, ceftiofur; ENRO, enrofloxacin; SXT, trimethoprim/sulfamethoxazole; OXY, oxytetracycline; and TET, tetracycline.

**Breakpoint reference: #1, CLSI VET01S (17); #2, Antimicrobial Therapy in Veterinary Medicine (19); and #3, NARMS (18).

Table 3. MIC (μg/ml) distribution (%) of *E. faecalis* isolates ($n = 20$).

Class	Agents*	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	MIC50	MIC90
Aminocoumarins	NOV				30	10	15	**45**									2	≥4
Aminocyclitols	SPE								5	0	55	**40**					32	**≥64**
Aminoglycosides	GEN				0	0	0	35	**65**								**>8**	**>8**
	NEO						0	5	10	30	**55**						**>32**	**>32**
	STR								0	5	30	15	30	0	0	**20**	64	**>1024**
Beta-lactams	AMOX			45	35	20	0	0	0	0							0.5	0.5
	XNL			5	5	0	1	**90**									**>4**	**>4**
	PEN	0	0	15	5	15	45	20	0								2	4
Florfenicols	FFN					30	65	5	0								2	2
Fluoroquinolones	ENRO		0	20	40	40	0										0.5	1
Lincosamides	CLI				10	5	5	**80**									**>4**	**>4**
Sulfonamides	SDM										0	0	0	**100**			**>256**	**>256**
	STZ										0	5	0	**95**			**>256**	**>256**
	SXT				100	0	0										≤0.5	≤0.5
Tetracyclines	OXY			45	45	10	0	0	0								0.5	0.5
	TET			50	45	5	0	0	0								≤0.25	0.5
Macrolides	ERY		60	5	5	25	5	0									≤0.12	0.25
	Agent						2.5	5	10	20							MIC50	MIC90
	TYLT						95	5	0	0							2.5	2.5

*Antimicrobial agents: SPE, spectinomycin; NOV, novobiocin; GEN, gentamicin; NEO, neomycin; STR, streptomycin; AMOX, amoxicillin; XNL, ceftiofur; PEN, penicillin; ENRO, enrofloxacin; CLI, clindamycin; FFN, florfenicol; SDM, sulphadimethoxine; STZ, sulphathiazole; SXT, trimethoprim/sulfamethoxazole; OXY, oxytetracycline; TET, tetracycline; ERY, erythromycin; and TYLT, tylosin tartrate. The shaded areas indicate concentrations not tested. Bold numbers indicate that isolates had MIC values greater than the highest concentration tested.

testing panel (AVIAN1F), which included 18 antibiotics belonging to 10 drug classes. Many of these antimicrobials are approved for use in poultry, according to the guidelines of the American Association of Avian Pathologists and American Veterinary Medical Association. One exception is enrofloxacin, a fluoroquinolone antibiotic that is illegal to use in poultry. Some of these antimicrobials are included in the NARMS panel, such as gentamicin, streptomycin, ceftiofur, sulfamethoxazole (prior to 2004), and tetracycline.

It was encouraging to see that all bobwhite *E. coli* isolates were susceptible to the following antibiotics: florfenicol, aminocyclitols (spectinomycin), aminoglycosides (gentamicin, neomycin, and streptomycin), beta-lactams (ceftiofur), fluoroquinolones (enrofloxacin), sulfonamides, combinations (trimethoprim/sulfamethoxazole), and tetracycline (oxytetracycline and tetracycline). Although all *E. coli* isolates showed high MICs (equal to or greater than the highest concentration tested) for clindamycin, penicillin, erythromycin, and tylosin, it is somewhat expected because *Enterobacteriaceae,* including *E. coli*, are intrinsically resistant to lincosamides, macrolides, and penicillin (10,13). However, reduced susceptibility to florfenicol and resistance to sulphathiazole and sulphadimethoxine were alarming. Florfenicol has been used to treat *E. coli* airsacculitis, and sulfonamides are routinely used as prophylactic or therapeutic agents to bacterial infections and coccidiosis in domestic poultry (1,11). Sulphathiazole was included in NARMS surveillance in 2003 and 2004 and then replaced by sulfisoxazole (8). NARMS data indicate that approximately 40% to 50% of *E. coli* isolates from retail poultry are resistant to sulphathiazole, figures that are higher than the resistance rate detected in bobwhite bacteria. Although the origin(s) of quail AMR is not known, the fact that more isolates were resistant to an older sulfonamide, sulfathiazole, than sulphadimethoxine suggests a selection pressure from antibiotic use in veterinary or human medicine. It is known that *Enterococcus* spp. have low-level intrinsic resistance to many clinically useful antimicrobials, such as sulfonamides, lincosamides, beta-lactams, and low-level aminoglycosides (12,22). In the present study, *E. faecalis* and *E. faecium* isolates had high MICs (equal to or greater than the highest concentrations included) to more than 50% of the antimicrobial agents tested. It is noteworthy that reduced susceptibility to erythromycin and resistance to high-concentration streptomycin were seen with quail enterococci and resistance to tetracycline was detected in one *E. faecium* isolate. A comparison of data on antimicrobials included in both NARMS and the present studies indicates that the MICs of bobwhite enterococci were lower than that of the retail poultry isolates. For example, the MIC_{90} of streptomycin against retail chicken *E. faecalis* isolates was 2048 μg/ml, whereas MIC_{90} for quail isolates was 64 μg/ml (6). The MICs of erythromycin were equal to or greater than 8 μg/ml in about 35% of retail chicken *E. faecalis* isolates, while MICs were 1 μg/ml for 100% of quail isolates (6).

The MIC panel (AVIAN1F) used in the present study includes most of the antimicrobials recommended for use in poultry by AAAP-AVMA, but the agents and test concentrations differ from that of NARMS or other previous studies conducted outside the United States (14). In addition, many studies of AMR in wild birds or poultry utilized the disk diffusion method or PCR detection of resistance genes (2,3,18), which make it impossible to compare the pattern or trend of AMR in different wild bird species. However, data from the present study revealed that AMR has already spread to the wild bobwhite populations in western Texas and western Oklahoma. It is noteworthy to point out that the study area is not known for domestic poultry production and has limited releases of

Table 4. MIC (μg/ml) distribution (%) of *E. faecium* isolates ($n = 10$).

Class	Agents*	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	MIC50	MIC90
Aminocoumarins	NOV				100	0	0	0									<=0.5	<=0.5
Aminocyclitols	SPE								0	0	10	**90**					**>64**	**>64**
Aminoglycosides	GEN				0	0	0	0	**100**								**>8**	**>8**
	NEO						0	0	0	10	**90**						**>32**	**>32**
	STR								0	0	0	10	0	0	0	**90**	**>1024**	**>1024**
Beta-lactams	AMOX			70	30	0	0	0	0	0							≤0.25	0.5
	XNL			0	0	0	0	**100**									**>4**	**>4**
	PEN	20	0	0	0	20	50	10	0								2	2
Florfenicols	FFN					0	70	30	0								2	4
Fluoroquinolones	ENRO		0	0	0	30	70										2	**≥2**
Lincosamides	CLI				0	0	0	**100**									**>4**	**>4**
Sulfonamides	SDM										0	0	0	**100**			**>256**	**>256**
	STZ										0	0	0	**100**			**>256**	**>256**
	SXT				0	0	**100**										**>2/38**	**>2/38**
Tetracyclines	OXY			40	40	10	0	0	**10**								0.5	1
	TET			60	30	0	0	0	**10**								0.25	0.5
Macrolides	ERY		20	0	10	20	50	0									1	2
	Agent						2.5	5	10	20							MIC50	MIC90
	TYLT						20	50	30	0							5	10

*Antimicrobial agents: SPE, spectinomycin; NOV, novobiocin; GEN, gentamicin; NEO, neomycin; STR, streptomycin; AMOX, amoxicillin; XNL, ceftiofur; PEN, penicillin; ENRO, enrofloxacin; CLI, clindamycin; FFN, florfenicol; SDM, sulphadimethoxine; STZ, sulphathiazole; SXT, trimethoprim/sulfamethoxazole; OXY, oxytetracycline; TET, tetracycline; ERY, erythromycin; and TYLT, tylosin tartrate. The shaded areas indicate concentrations not tested. Bold numbers indicate that isolates had MIC values greater than the highest concentration tested.

pen-reared bobwhite birds. Findings from the present study warrant additional testing of wild and pen-reared bobwhite birds in different regions and the appropriate environmental samples to understand the origin and transmission of AMR in pen-reared and wild bobwhite populations. It is also important to increase the awareness of quail AMR to the general public.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Steve Presley, Anna Gibson, and Kristan Urban with the Central Receiving Lab at Texas Tech University for their assistance with sample procurement. This work was financially supported by the Rolling Plains Quail Research Foundation. Drs. Michael Zhang, Shuping Zhang, and Dale Rollins conceived the project. Drs. Michael Zhang and Shuping Zhang designed the experiments. Drs. Michael Zhang, Zhenyu Shen, and William Fales conducted experiments and analyzed data. Drs. Shuping Zhang and Michael Zhang drafted the manuscript.

REFERENCES

1. Afifi, N. A., and K. A. El-Sooud. Tissue concentrations and pharmacokinetics of florfenicol in broiler chickens. Dtsch. Tierarztl. Wochenschr. 104:178–180. 1997.

2. Bonnedahl, J., P. Drobni, A. Johansson, J. Hernandez, A. Melhus, J. Stedt, B. Olsen, and M. Drobni. Characterization, and comparison, of human clinical and black-headed gull (*Larus ridibundus*) extended-spectrum beta-lactamase-producing bacterial isolates from Kalmar, on the southeast coast of Sweden. J. Antimicrob. Chemother. 65:1939–1944. 2010.

3. Carroll, D., J. Wang, S. Fanning, and B. J. McMahon. Antimicrobial resistance in wildlife: implications for public health. Zoonoses Public Health 62:534–542. 2015.

4. Catry B., H. Laevens, L. A. Devriese, G. Opsomer, and A. De Kruif. Antimicrobial resistance in livestock. J. Vet. Pharmacol. Ther. 26:81–93. 2003.

Table 5. Antimicrobial susceptibility pattern of *Enterococcus* isolates based on MIC breakpoints for veterinary pathogens.

		Breakpoints				% *E. faecalis* ($n = 20$)			% *E. faecium* ($n = 10$)		
Class	Antimicrobials*	S	I	R	Reference**	S	I	R	S	I	R
Aminocoumarins	NOV	≤4	8	>16	#2	55	0	**45**	100	0	0
Aminoglycosides	STR	≤512	—	≥1024	#3	80	—	**20**	20	—	**80**
Beta-lactams	PEN	≤8	—	≥16	#1	100	—	0	100	—	0
Macrolides	ERY	≤0.5	1–4	≥8	#1	70	**30**	0	30	**70**	0
Macrolides	TYLT	≤5	10	≥20	#2	100	0	0	100	0	0
Tetracyclines	OXY	≤4	8	≥16	#1	100	0	0	90	0	**10**
	TET	≤4	8	≥16	#1	100	0	0	90	0	**10**

*Antimicrobial agents: NOV, novobiocin; STR, streptomycin; PEN, penicillin; ERY, erythromycin; TYLT, tylosin tartrate; OXY, oxytetracycline; TET, tetracycline.

**Breakpoint reference: #1, CLSI (17); #2, Antimicrobial Therapy in Veterinary Medicine (19); and #3, NARMS (18).

5. [CDC] Centers for Disease Control. Antibiotic resistance threats in the United States. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta. 2013.

6. CDC. National antimicrobial resistance monitoring system for enteric bacteria (NARMS): retail meat report, 2011. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta. 2013.

7. [CLSI] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals. CLSI Document VET01S, 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA. 2015.

8. CLSI. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. CLSI Document M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA. 2007.

9. DeVos, T., and D. W. Speake. Effects of releasing pen-raised northern bobwhites on survival rates of wild populations of northern bobwhites. Wildl. Soc. Bull. 23:267–273. 2005.

10. Frye, J. G., and C. R. Jackson. Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica*, *Escherichia coli*, and *Enterococcus* spp. isolated from U.S. food animals. Front. Microbiol. 4:135. 2013.

11. Giguère, S., J. F. Prescott, J. D. Baggot, R. D. Walker, and P. M. Dowling. eds. Antimicrobial therapy in veterinary medicine, 4th ed. Blackwell Publishing, Oxford, UK. 2013.

12. Hollenbeck, B. L., and L. B. Rice. Intrinsic and acquired resistance mechanisms in *Enterococcus*. Virulence 3:421–433. 2012.

13. Leclercq, R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin. Infect. Dis. 34:482–492. 2002.

14. Maasjost, J., K. Mühldorfer, S. Cortez de Jäckel, and H. M. Hafez. Antimicrobial susceptibility patterns of *Enterococcus faecalis* and *Enterococcus faecium* isolated from poultry flocks in Germany. Avian Dis. 59:143–148. 2015.

15. Mølbak, K. Spread of resistant bacteria and resistance genes from animals to humans—the public health consequences. J. Vet. Med. B Infect. Dis. Vet. Public. Health 51:364–369. 2004.

16. Nelson, M., S. H. Jones, C. Edwards, and J. C. Ellis. Characterization of *Escherichia coli* populations from gulls, landfill trash, and wastewater using ribotyping. Dis. Aquat. Organ. 81:53–63. 2008.

17. Oravcova, V., L. Zurek, A. Townsend, A. B. Clark, J. C. Ellis, A. Cizek, and I. Literak. American crows as carriers of vancomycin-resistant enterococci with vanA gene. Environ. Microbiol. 16:939–949. 2014.

18. Pinto, L., H. Radhouani, C. Coelho, P. Martins da Costa, R. Simões, R. M. Brandão, C. Torres, G. Igrejas, and P. Poeta. Genetic detection of extended-spectrum beta-lactamase-containing *Escherichia coli* isolates from birds of prey from Serra da Estrela Natural Reserve in Portugal. Appl. Environ. Microbiol. 76:4118–4120. 2010.

19. Poeta, P., H. Radhouani, G. Igrejas, A. Gonçalves, C . Carvalho, J. Rodrigues, L. Vinué, S. Somalo, and C. Torres. Seagulls of the Berlengas natural reserve of Portugal as carriers of fecal *Escherichia coli* harboring CTX-M and TEM extended-spectrum beta-lactamases. Appl. Environ. Microbiol. 74:7439–7441. 2008.

20. Radhouani, H., P. Poeta, A. Gonçalves, R. Pacheco, R. Sargo, and G. Igrejas. Wild birds as biological indicators of environmental pollution: antimicrobial resistance patterns of *Escherichia coli* and enterococci isolated from common buzzards (*Buteo buteo*). J. Med. Microbiol. 61:837–843. 2012.

21. Radhouani, H. N. Silva, P. Poeta, C. Torres, S. Correia, and G. Igrejas. Potential impact of antimicrobial resistance in wildlife, environment and human health. Front. Microbiol. 5:23. 2014.

22. Rice, L. B. Mechanisms of resistance and clinical relevance of resistance to β-lactams, glycopeptides, and fluoroquinolones. Mayo Clin. Proc. 87:198–208. 2012.

23. Santos, T., N. Silva, G. Igrejas, P. Rodrigues, J. Micael, T. Rodrigues, R. Resendes, A. Gonçalves, C. Marinho, D. Gonçalves, R. Cunha, and P. Poeta. Dissemination of antibiotic resistant *Enterococcus* spp. and *Escherichia coli* from wild birds of Azores Archipelago. Anaerobe 24:25–31. 2013.

24. Sato, G., C. Oka, M. Asagi, and N. Ishiguro. Detection of conjugative R plasmids conferring chloramphenicol resistance in *Escherichia coli* isolated from domestic and feral pigeons and crows. Zentralbl. Bakteriol. Orig. A 241:407–417. 1978.

25. Sjölund, M., J. Bonnedahl, J. Hernandez, S. Bengtsson, G. Cederbrant, J. Pinhassi, G. Kahlmeter, and B. Olsen. Dissemination of multidrug-resistant bacteria into the Arctic. Emerg. Infect. Dis. 14:70–72. 2008.

26. Smith, S., J. Wang, S. Fanning, and B. J. McMahon. Antimicrobial resistant bacteria in wild mammals and birds: a coincidence or cause for concern? Ir. Vet. J. 67:8. 2014.

27. Su, H., J. McKelvey, D. Rollins, M. Zhang, D. J. Brightsmith, J. Derr, and S. Zhang. Cultivable bacterial microbiota of northern bobwhite (*Colinus virginianus*): a new reservoir of antimicrobial resistance? PLoS One 9:e99826. 2014.

28. Texas Quail Study Group. Quail restoration in the Post Oak Savanna, Blackland, and Coastal Prairies. In: Proc. 2009 Texas Quail Study Group, Cat Spring, TX. 2009.

29. Threlfall, E. J., L. R. Ward, J. A. Frost, and G. A. Willshaw. The emergence and spread of antibiotic resistance in food-borne bacteria. Int. J. Food Microbiol. 62:1–5. 2000.

30. U.S. Fish & Wildlife Service. National survey of fishing, hunting, and wildlife-associated recreation: national overview. U.S. Fish & Wildlife Service, Washington, DC. 2011.

31. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. Pharm. Therap. 40:277–283. 2015.