NRC Research Press

ARTICLE

In vitro transduction of antimicrobial resistance genes into *Escherichia coli* isolates from backyard poultry in Mexico

Juan Martín Talavera-González, Martín Talavera-Rojas, Edgardo Soriano-Vargas, Jesús Vázquez-Navarrete, and Celene Salgado-Miranda

Abstract: The transmission of multidrug-resistant pathogens and antimicrobial resistance genes is an emerging problem involving multiple factors (humans, domestic animals, wildlife). The aim of this study was to investigate the presence of *Escherichia coli* isolates with different antimicrobial resistance genes from backyard poultry and to demonstrate the in vitro transduction phenomenon of these genes between phages from migratory wild birds and poultry *E. coli* isolates. We collected 197 *E. coli* isolates from chickens, turkeys, and ducks in backyard production units (northern region of the State of Mexico). Isolates were resistant to ampicillin (80.7%), tetracycline (64.4%), carbenicillin (56.3%), and nalidixic acid and trimethoprim–sulfamethoxazole (both, 26.9%). Moreover, the genes bla_{TEM} (56.3%), *tetB* (20.8%), *tetA* (19.2%), *sulI* (7.6%), *sulII* (10.1%), *qnrA* (9.6%), and *qnrB* (5.5%) were found. In vitro transduction using phages from migratory wild birds sampled in the wetland Chimaliapan (State of Mexico) was successfully achieved. It was possible to transduce *qnrA*, *tetB*, bla_{TEM}, and *sulII* genes to *E. coli* isolates from poultry. This is the first report that describes the transduction of antimicrobial resistance genes from phages of migratory wild birds to poultry and suggests the possible transmission in backyard production units.

Key words: backyard poultry, antimicrobial resistance, bacteriophages, transduction, wild birds.

Résumé : La transmission d'agents pathogènes multirésistants et de gènes de résistance aux antimicrobiens est un problème en émergence impliquant de multiples facteurs (humains, animaux domestiques, faune). L'objectif de cette étude était d'étudier la présence d'isolats d'*Escherichia coli* comportant différents gènes de résistance aux antimicrobiens provenant de volailles urbaines et de démontrer le phénomène de transduction *in vitro* de ces gènes entre les phages d'oiseaux sauvages migrateurs et les isolats d'*E. coli* de volaille. Les auteurs ont récolté 197 isolats d'*E. coli* de poulets, de dindes et de canards dans des unités de production urbaines (région nord de l'État de Mexico). Les isolats étaient résistants à l'ampicilline (80,7 %), à la tétracycline (64,4 %), à la carbénicilline (56,3 %), ainsi qu'à l'acide nalidixique et au triméthoprime–sulfaméthoxazole (26,9 % chacun). De plus, les gènes bla_{TEM} (56,3 %), *tetB* (20,8 %), *tetA* (19,2 %), *sulI* (7,6 %), *sulII* (10,1 %), *qnrA* (9,6 %) et *qnrB* (5,5 %) ont été trouvés. La transduction in vitro à l'aide des phages d'oiseaux sauvages migrateurs échantillonnés dans la zone humide de Chimaliapan (État de Mexico) a été réalisée avec succès. Il a été possible de transduire les gènes *qnrA*, *tetB*, bla_{TEM} et *sulII* à des isolats d'*E. coli* provenant de volailles. Il s'agit du premier rapport qui décrit la transduction des gènes de résistance aux antimicrobiens des phages des oiseaux sauvages migrateurs aux volailles et suggère la transmission possible dans les unités de production urbaines. [Traduit par la Rédaction]

Mots-clés : volailles urbaines, résistance aux antimicrobiens, bactériophages, transduction, oiseaux sauvages.

Introduction

Poultry production is one of the most important industries worldwide. In Mexico, poultry foods are part of the diet of 60% of the population and contribute up to 55% of the animal protein intake (CEDRSSA 2019). Although the commercial poultry industry is the main supplier in Mexico, backyard poultry farms are very common throughout the country in rural and peri-urban regions. Both production systems are prone to infections by *Escherichia coli* or other pathogens that could inflict economic losses; hence, the implementation

Received 8 June 2020. Revision received 1 December 2020. Accepted 21 December 2020.

J.M. Talavera-González, M. Talavera-Rojas, E. Soriano-Vargas, and C. Salgado-Miranda. Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Carretera Toluca-Atlacomulco, Km 15.5, Toluca, Estado de México 50200, México.

J. Vázquez-Navarrete. Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Carretera México-Toluca Km. 15.5, Ciudad de México, México.

Corresponding author: Martín Talavera-Rojas (email: talaverarojas@gmail.com).

of antimicrobial therapy is necessary in several cases (Ahmed et al. 2013).

While commercial farms have well-established vaccination programs and guidelines that lessen the impact of the disease, backyard poultry farms usually lack veterinary supervision, driving the mishandling of antibiotics. The gut microbiota is then subjected to selective pressure that can lead to the acquisition of antimicrobial resistance genes (ARGs) and consequently the development of multidrug-resistant (MDR) microorganisms, including *E. coli* (van den Bogaard et al. 2001). These microorganisms are evacuated daily through feces that are washed away by rain from rural households that lack domestic waste disposal systems for excreta, promoting pollution of aquatic environments like rivers, lagoons, or wetlands, which are places where multiple species coexist, and among them, wild birds stand out as vectors responsible for disseminating pathogens to distant regions (Sedeño-Díaz and López-López 2007; Alcalá et al. 2016).

During the migration phenomenon, over 20 wild bird species arrive in Mexico; a recent study reported MDR *E. coli* isolates from some wild birds in the wetlands of Lerma, State of Mexico (Ramírez-Martínez et al. 2018). These wild birds travel in rural and peri-urban regions with crops, pastures, and backyard production units, where they can have direct contact with poultry and other domestic animals (Parker et al. 2016). In Mexico, antimicrobial resistance (AMR) to antibiotics, ARGs, and virulence factors from pathogens in the poultry industry have been described; nonetheless, there is a knowledge gap about the frequency of multidrug resistance linked to ARGs in backyard poultry in rural and peri-urban regions.

When there are interactions between different animal species and humans, the risk of transmission of pathogens and development of infectious illness is high; moreover, the swap of virulence and ARGs through mobile genetic elements like bacteriophages, plasmids, and transposons could rearrange the genetic structure of the normal microbiota. In vitro transduction by bacteriophages has been reported in different investigations with bacterial isolates from animals and humans capable of exchanging genetic material (even among microorganisms of different genera); nevertheless, there is no transduction evidence between bacterial isolates from migratory wild birds and poultry (García-Aljaro et al. 2006).

This work aimed to describe the frequency of AMR and ARGs in poultry from rural and peri-urban areas in the northern region of the State of Mexico and to evaluate the in vitro transduction between *E. coli* isolates from migratory birds and backyard poultry to appraise the potential risk of transmission when these species interact.

Materials and methods

Sample collection in poultry

A total of 322 cloacal samples were collected from apparently healthy poultry (chickens, 214 samples; turkeys, 54 samples; and ducks, 54 samples) in backyard production units of rural and peri-urban areas from 4 municipalities: Atlacomulco (81 samples), El Oro (80 samples), Jocotitlán (80 samples), and Ixtlahuaca (81 samples), located in the northern region of the State of Mexico, Mexico.

Sample collection in wild birds

From July (2017) through March (2018), a total of 63 cloacal samples were opportunistically collected from hunter-harvested wild birds. Samples belonged to 9 duck species: *Anas crecca* (6), *Mareca americana* (5), *Anas diazi* (6), *Mareca strepera* (12), *Anas acuta* (4), *Spatula discors* (10), *Oxyura jamaicensis* (2), *Spatula clypeata* (12), *Spatula cyanoptera* (3), and 3 unidentified species. Samples were individually collected in sterile tubes with Stuart media and stored at 4 °C and transported to the laboratory. The study area was Ciénega Chimaliapan, which is highly polluted with sewage and belongs to the system of wetlands Ciénegas of Lerma located in the State of Mexico, Mexico (9°14′N, 99°30′W) with an approximate distance of ∼85 km from Atlacomulco, 113 km from El Oro, 56 km from Ixtlahuaca, and 73 km from Jocotitlán, where backyard production units were sampled.

Isolation and identification of *E. coli*

Samples were collected with a cloacal swab and carried to the Centro de Investigación y Estudios Avanzados en Salud Animal in Stuart transport medium at 4 °C. Each sample was plated onto MacConkey agar and incubated at 37 °C for 24 h. Colonies with morphology similar to *E. coli* were subjected to biochemical tests (triple sugar iron agar, lysine iron agar, sulfide indole motility, and Simmons citrate agar). Finally, identification was confirmed by amplification of the *uidA* gene with endpoint PCR (Aguilar-Montes de Oca et al. 2015).

Antimicrobial susceptibility

A Kirby–Bauer test was carried out according to the Clinical and Laboratory Standard Institute (CLSI 2012). The selected antimicrobial discs were ampicillin 10 μg (AMP), amikacin 30 μg (AMK), carbenicillin 100 μg (CAR), gentamicin 10 μg (GEN), cephalothin 30 μg (CEF), cefotaxime 30 μg (CTX), netilmicin 30 μg (NET), ciprofloxacin 5 μg (CIP), norfloxacin 10 μg (NOR), chloramphenicol 30 μg (CHL), trimethoprim–sulfamethoxazole 25 μg (SXT), nitrofurantoin 300 μg (NIT), nalidixic acid 30 μg (NAL), and tetracycline 30 μg (TET). The CLSI guidelines for control strain (*E. coli* ATCC 25922), interpretation, and classification were followed. Isolates were classified as MDR when AMR to ≥3 antibiotics classes was detected (CLSI 2012, 2015).

Table 1. Primers used in this study.

Gene	Primer name	Sequence (5′–3′)	Amplicon size (bp)	Reference
bla_{TEM}	MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	800	Dallenne et al. 2010
	MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC		
qnrA	QnrAm_F	AGAGGATTTCTCACGCCAGG	580	Kraychete et al. 2016
	QnrAm_R	TGCCAGGCACAGATCTTGAC		
qnrB	QnrBm_F	GGMATHGAAATTCGCCACTG	264	
	QnrBm_R	TTTGCYGYYCGCCAGTCGAA		
tetA	Tet (A)-F	GTGAAACCCAACATACCCC	888	Hedayatianfard et al. 2014
	Tet (A)-R	GAAGGCAAGCAGGATGTAG		
tetB	Tet (B)-F	CCTTATCATGCCAGTCTTGC	774	
	Tet (B)-R	ACTGCCGTTTTTTCGCC		
sulI	Sul 1-F	CGGCGTGGGCTACCTGAACG	433	Kerrn et al. 2002
	Sul 1-B	GCCGATCGCGTGAAGTTCCG		
sulII	Sul 2-F	GCGCTCAAGGCAGATGGCATT	293	
	Sul 2-B	GCGTTTGATACCGGCACCCGT		
uidA	UAL1939b	ATGGAATTTCGCCGATTTTGC	187	Heijnen and Medema 2006
	UAL2105b	ATTGTTTGCCTCCCTGCTGC		

Detection of ARGs

PCR was used to confirm gene presence in bacteriophages and donor and recipient strains. Primers and amplification conditions are listed in Table 1. All samples were screened with a multiplex PCR to detect AMR to quinolones (*qnrA* and *qnrB* genes), tetracyclines (*tetA* and *tetB*), and sulfonamides (*sulI* and *sulII*), and with a PCR to detect bla_{TEM}. Amplicons were visualized in a UV transilluminator after running at 75 V for 1 h on a 2% agarose gel containing ethidium bromide (0.05 mg/L).

Selection of donor and recipient isolates

Four MDR *E. coli* isolates from wild birds with the presence of several ARGs (D1, D11, D21, and D22) were selected to be donor isolates and obtain the phages. Fourteen isolates from wilds birds and 13 *E. coli* isolates from domestic poultry (free of phenotypic and molecular resistance to the antibiotics and free of the ARGs screened) were selected to be used as recipient isolates. Of the *E. coli* isolates from backyard poultry, 9 were from the municipality of Ixtlahuaca (2 from chickens, 1 from turkey, and 6 from ducks) and 4 were from El Oro (from chickens).

Prophage induction and identification of recipient isolates

Three serial dilutions of donor isolates were prepared (1/10, 1/100, and 1/1000) in tryptic soy broth (TSB) and incubated at 37 °C for 3–4 h. Next, a 5 μL aliquot of each dilution was placed on Petri dishes with tryptic soy agar (TSA) and incubated for 4 h at 37 °C. Afterwards, the Petri dishes were irradiated with UV light (320 nm) for 10–15 s, and immediately 3 mL of soft agar (supplemented with 0.3 mL of a 5 mol/L $MgCl_2$ solution and 0.2 mL of a 2 mol/L $CaCl_2$ solution) plus 0.5 mL of the recipient strains in exponential phase was poured in the plates and the plates were then incubated at 37 °C for 24 h. Finally, the Petri dishes were examined for bacteriophage lysis (Sekulović and Fortier 2016).

Phage purification and amplification

Only one lysis area of each identified recipient isolate was collected with a pipette tip and transferred to 0.5 mL of TSB supplemented with 10 mmol/L $MgCl_2$ and 10 mmol/L $CaCl_2$ and kept at room temperature for 3 h. Later, the supernatant was filtered and serially diluted (up to 10^{-4}) with 0.5 mL of TSB. Subsequently, an aliquot (100 μL) of each dilution and the receptor isolate (100 μL) were mixed in soft agar and poured in TSA, followed by incubation at 37 °C for 24 h. This purification process was repeated at least 3 times to ensure the purification of each phage (Sekulović and Fortier 2016). To obtain high titer stocks of phage, 6–7 plaques and 100 mg of receptor isolate were mixed in 10 mL of TSB supplemented with 10 mmol/L $MgCl_2$ + 10 mmol/L $CaCl_2$ and incubated at 37 °C for 5–7 h until visual bacterial lysis was observed, followed by filtration. Afterward, 50 μL of this first phage lysate was freshly mixed with 100 μL of recipient isolate and incubated at 37 °C for 5–7 h and filtered; a phage titration was performed before storage (Fortier and Moineau 2009).

Bacteriophage titration

A mix of 100 μL of each recipient isolate and 100 μL of the phage lysate (10^{-1} to 10^{-6}) was made in soft agar and poured in TSA plates, followed by incubation at 37 °C for 24 h.

The titer range was calculated and expressed as plaque-forming units per millilitre (PFU/mL) (Kropinski et al. 2009). The values ranged from ~3.8×10^3 to 6.7×10^5 PFU/mL.

Extraction and purification of bacteriophage DNA

DNase (1 μL) and RNase (1 μL) were added to 1 mL of phage lysate and incubated at 37 °C for 30 min to

Table 2. Antimicrobial resistance of *Escherichia coli* isolates by municipality in the State of Mexico.

	% Antimicrobial resistance (no. of isolates) in the municipalities of:				
Antibiotic	Atlacomulco (n = 73)	El Oro (n = 44)	Ixtlahuaca (n = 30)	Jocotitlán (n = 50)	Total (n = 197)
AMK	13.7 (10)	4.6 (2)	6.7 (2)	—	7.1 (14)
AMP	82.2 (60)	72.7 (32)	60 (18)	98 (49)	80.7 (159)
CAR	45.2 (33)	43.1 (19)	63.3 (19)	80 (40)	56.3 (111)
CEF	5.4 (4)	11.3 (5)	26.6 (8)	6 (3)	10.1 (20)
CTX	—	—	3.3 (1)	2 (1)	1 (2)
CIP	9.5 (7)	9 (4)	43.3 (13)	12 (6)	15.2 (30)
CHL	16.4 (12)	11.3 (5)	30 (9)	12 (6)	16.2 (32)
GEN	5.4 (4)	—	16.6 (5)	6 (3)	6 (12)
NET	1.3 (1)	6.8 (3)	13.3 (4)	2 (1)	4.5 (9)
NIT	10.9 (8)	2.2 (1)	—	4 (2)	5.5 (11)
NOR	6.8 (5)	9 (4)	46.6 (14)	14 (7)	15.2 (30)
SXT	28.7 (21)	22.7 (10)	36.6 (11)	22 (11)	26.9 (53)
NAL	23.2 (17)	20.4 (9)	56.6 (17)	20 (10)	26.9 (53)
TET	73.9 (54)	68.1 (30)	66.6 (20)	46 (23)	64.4 (127)
CAZ	—	—	3.3 (1)	—	0.5 (1)

Note: AMK, amikacin; AMP, ampicillin; CAR, carbenicillin; CEF, cephalothin; CTX, cefotaxime; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; NET, netilmicin; NIT, nitrofurantoin; NOR, norfloxacin; SXT, trimethoprim–sulfamethoxazole; NAL, nalidixic acid; TET, tetracycline; CAZ, ceftazidime.

remove bacterial DNA, followed by the addition of proteinase K (10 μL) and incubation for 10 min at 37 °C; then 0.1 mL of SDS was added and incubated at 65 °C for 30 min. The supernatant was mixed manually with an equivalent volume of phenol–chloroform–isoamyl alcohol (25:24:1) at room temperature and centrifugated for 10 min at 13 000*g*. The aqueous phase was mixed with isopropanol and the pellet was washed with ethanol (70%) and centrifuged at 13 000*g* for 5 min; this washing process was repeated two more times. Finally, the pellet was suspended in 50 μL of 10 mmol/L Tris–HCl (Sekulović and Fortier 2016). To confirm the removal of bacterial DNA, a PCR with the *uidA* gene was carried out (Colomer-Lluch et al. 2014*a*). The purity and concentration of DNA were determined with a nanodrop spectrophotometer (Quawell q5000) at an absorbance of 260 nm.

In vitro transduction

A mix of 10 μL of recipient strains (overnight culture) and 10 μL of phage lysate was incubated for 30 min at 37 °C. TSB was added to the mix and then incubated with shaking for 2 h. TSA was centrifuged at 12 000 *g* for 3 min, the supernatant was discarded and the pellet was resuspended in 150 μL of Mueller–Hinton broth and poured in Mueller–Hinton agar (Shousha et al. 2015). A Kirby–Bauer test was performed according to the CLSI (2012). In addition, the genes were confirmed through PCR as described in the screening section of Materials and methods.

Results

Isolation and identification of *E. coli* from poultry

Sixty-one percent of the *E. coli* isolates in this study were collected from poultry. The highest number of isolates were from the municipality of Atlacomulco (37%), followed by Jocotitlán (25.3%), El Oro (22.3%), and Ixtlahuaca (15.2%). The frequency of *E. coli* isolates in poultry species was 81.2% for chickens, 13.2% for ducks, and 5.6% for turkeys.

Antimicrobial susceptibility

Most of the isolates were resistant to AMP (80.7%), followed by TET (64.4%), CAR (56.3%), and finally NAL and SXT (both 26.9%) (Table 2). With respect to poultry species, 78.1% of *E. coli* isolates from chicken showed antimicrobial resistance to AMP, followed by TET (68.7%), SXT (26.8%), and NAL (25%). Similarly, 100% of *E. coli* isolates from turkey were resistant to AMP, followed by TET (63.3%), NAL (63.6%), and SXT (27.2%). In the case of isolates from duck, 69.2% were resistant to AMP, 38.4% to TET, 26.9% to SXT, and 23% to NAL (Table 2). Multiresistance was found in 66.6% of isolates from Ixtlahuaca, 58.8% from Atlacomulco, 50% from Jocotitlán, and 45.5% from El Oro.

Detection of ARGs in *E. coli* isolates from poultry

Genes related to AMR against quinolones, sulfonamides, tetracyclines, and β-lactamics were found. The bla_{TEM} gene was the most frequent ARG (56.3%), followed by *tetB* (20.8%), *tetA* (19.2%), *sulII* (10.1%), *qnrA* (9.6%), *sulI* (9.5%), and finally *qnrB* (5.5%) (Table 3).

Bacteriophage isolation

Four bacteriophages were isolated from MDR bacterial isolates collected from migratory birds (*Anas crecca*, *Anas acuta*, *Oxyura jamaicensis*, and *Spatula discors*) (Table 4). The bacteriophages titer ranged from 3.8×10^5 to 6.7×10^6 PFU/mL, and four ARGs were detected (Table 5).

Transduction

Phages were able to infect a total of 8/13 (61.5%) nonresistant *E. coli* isolates from backyard poultry, 3 belonged to the municipality of El Oro (chicken) and 5 to Ixtlahuaca (1 from chicken and 4 from ducks), and a

Table 3. Detected genes in poultry according to municipalities and species.

	No. of isolates expressing gene in the municipality of:											
	Atlacomulco			El Oro			Ixtlahuaca			Jocotitlán		
Gene	CK	TY	DU	CK	TY	DU	CK	TY	DU	CK	TY	DU
bla_{TEM}	55	–	–	20	–	–	9	4	3	15	2	3
tetA	21	–	–	9	–	–	3	–	4	1	1	1
tetB	30	–	–	7	–	–	1	1	2	3	1	1
qnrA	8	–	–	8	–	–	1	–	–	1	–	1
qnrB	5	–	–	1	–	–	–	1	–	3	–	1
sulI	10	–	–	6	–	–	–	1	–	–	–	–
sulII	10	–	–	4	–	–	–	1	–	3	1	–

Note: CK, chicken; TY, turkey; DU, duck.

Table 4. Bacteriophage origin and phenotypic and genotypic profile of *Escherichia coli* isolates obtained from migratory wild birds.

		Susceptibility test														ARGs						
ID	Species	AMK	AMP	CAR	CEF	CTX	CIP	CHL	GEN	NET	NIT	NOR	SXT	NAL	TET	*sulI*	*sulII*	*tetA*	*tetB*	bla_{TEM}	*qnrA*	*qnrB*
D1	*Anas crecca**	S	S	R	R	S	I	S	S	S	S	S	S	R	R	–	–	–	+	+	+	–
D2	*Anas diazi*	S	I	I	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D3	*Mareca Americana*	S	R	R	S	S	I	S	S	S	S	S	S	I	R	–	–	+	–	+	–	–
D4	Unknwon	S	R	R	R	I	S	S	S	S	S	S	S	S	S	–	–	–	–	+	–	–
D5	*Mareca strepera*	S	S	I	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D6	*Anas acuta*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D7	*Mareca strepera*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D8	*Spatula clypeata*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D9	*Spatula cyanoptera*	S	I	I	I	S	S	S	S	S	S	S	S	S	I	–	–	–	–	+	–	–
D10	*Spatula clypeata*	S	S	I	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D11	*Anas acuta**	S	S	I	S	S	R	S	R	S	S	R	R	R	R	+	+	+	–	+	+	–
D12	*Anas acuta*	S	I	I	S	S	S	S	S	S	S	S	S	S	I	–	–	–	–	–	–	–
D13	*Spatula cyanoptera*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	–	–	–	–	–	–	–
D14	*Spatula discors*	S	S	I	S	S	S	S	S	S	S	S	S	S	I	–	–	–	–	–	–	–
D15	*Spatula discors*	S	S	I	I	S	S	S	S	S	S	S	S	S	I	–	–	–	–	–	–	–
D16	*Spatula discors*	S	S	I	I	S	S	S	S	S	S	S	S	S	I	–	–	–	–	–	–	–
D17	*Spatula discors*	S	R	R	S	S	S	S	S	S	S	S	S	S	I	–	–	–	–	+	–	–
D18	*Spatula clypeata*	S	R	R	S	S	S	S	S	S	S	S	S	I	S	–	–	–	–	–	–	–
D19	*Mareca strepera*	S	R	R	I	S	S	S	S	S	S	S	S	S	I	–	–	–	–	+	–	–
D20	*Anas crecca*	S	R	R	I	S	S	S	S	S	S	S	S	S	S	–	–	–	–	+	–	–
D21	*Oxyura jamaicensis**	I	R	R	R	S	R	R	R	S	S	R	R	R	R	–	+	+	–	+	+	–
D22	*Spatula discors**	S	R	R	S	S	R	R	R	S	S	R	R	R	R	–	+	–	+	+	–	+
D23	*Oxyura jamaicensis*	S	S	I	S	S	S	S	S	S	I	S	S	S	S	–	–	–	–	–	–	–
D24	*Anas diazi*	S	S	I	S	S	S	S	S	S	S	S	S	S	I	–	–	–	–	–	–	–
Resistance (%)		0	30.7	34.6	11.5	0	11.5	7.6	11.5	0	0	11.5	11.5	15.3	19.2	4.1	12.5	12.5	8.3	41.6	12.5	4.1

Note: AMK, amikacin; AMP, ampicillin; CAR, carbenicillin; CEF, cephalothin; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; ID, identification; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; TET, tetracycline. S, sensitive; I, intermediate; R, resistant; ARGs, antimicrobial resistance genes; +, gene present; –, gene absent.

*Bird species from which phages were isolated.

Table 5. Origin and characteristics of phage isolates.

E. coli isolate (ID)	Bird species	Phage isolate (ID)	Phage titer (PFU/mL)	ARGs
D1	*Anas crecca*	T1	4.2×10^5	*tetB*
D11	*Anas acuta*	T11	6.5×10^6	*qnrA*
D21	*Oxyura jamaicensis*	T21	3.8×10^5	*qnrA*, *sulII*
D22	*Spatula discors*	T22	6.7×10^6	*tetB*, bla_{TEM}

Note: ID, identification. ARGs, antimicrobial resistance genes.

Table 6. Lysogenic conversion of antimicrobial resistance genes (ARGs) and phenotypic expression.

Phage ID	Phenotypic AMR transduced	Transduced ARGs	Infected *E. coli* isolates from backyard poultry	Infected *E. coli* isolates (ID) from wild birds
T1	CAR, TET	*tetB*	CK_a	D5, D12, D16
T11	GEN, NAL	*qnrA*	CK_a, DU_b, and CK_b	D2, D8, D10, D24
T21	SXT, NAL	*sul2* + *qnrA*	DU_b (2 isolates)	D15, D23
T22	AMP, TET	*tetB* + bla_{TEM}	CK_a and DU_b	D6, D7

Note: ID, identification; AMR, antimicrobial resistance; CAR, carbenicillin; TET, tetracycline; GEN, gentamicin; NAL, nalidixic acid; SXT, trimethoprim–sulfamethoxazole; AMP, ampicillin; CK_a, chicken isolate from El Oro; CK_b, chicken isolate from Ixtlahuaca; DU_b, duck isolate from Ixtlahuaca.

total of 11/14 (78.5%) non-resistant *E. coli* isolates from 6/9 (66.6%) wild birds species. Lysogenic conversion of 3/13 (23%) and 4/14 (28.5%) *E. coli* isolates from poultry and wild birds, respectively, were observed with phage T11, while phages T21 and T22 were able to infect 4/13 (30.7%) isolates from poultry and 4/14 (28.5%) from wild birds and expressed *sulII*, *qnrA*, *tetB*, and bla_{TEM} genes. Finally, the lysogenic conversion was detected in 1/13 (7.6%) *E. coli* isolates from poultry and 3/14 (21.4%) with *tetB* gene expressed (Table 6).

Discussion

Antimicrobial resistance has become a relevant focus in human and veterinary medicine. Overdependence on frequently used antibiotics in therapy and prophylaxis of infectious diseases and as growth promoters in livestock has caused a selective pressure in the animals' gut microbiota (Okpara et al. 2018; Koga et al. 2015).

Escherichia coli is usually considered a beneficial commensal of the gastrointestinal tract in animals; nonetheless, some strains of this microorganism can be pathogenic and are responsible for colibacillosis in the poultry industry (van Hoek et al. 2016). In this work, we collected 197 *E. coli* isolates from rural and peri-urban areas where poultry is in direct or indirect contact with other animal species and humans. In similar studies, MDR *E. coli* isolates were widespread in backyard production units. For example, Okpara et al. (2018) found 49 extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates from backyard animals including poultry in Nigeria, while Armas-Freire et al. (2015) reported 246 *E. coli* isolates from poultry in a rural area from Ecuador with a high frequency of AMR to quinolones. A mix of several species including pigs, chickens, turkeys, ducks, sheep, cattle, or horses are observed in backyard production units; these animals are usually employed as self-consumption or as a secondary economic income. In general, these production units did not have adequate biosecurity measures; moreover, poor regulation of the sale of antibiotics and restricted access to veterinary services can boost an inadequate use of clinically relevant drugs by unqualified staff, triggering the emergence of MDR bacteria (Amin et al. 2020; Okpara et al. 2018).

The AMR frequencies to AMP reported by Kissinga et al. (2018) in domestic ducks and backyards flocks in Tanzania were similar to those of our study (81.3% and 80.7%, respectively). In contrast, they found higher resistance than our study to SXT (75.8% and 26.9%, respectively) and CFX (62.3% in their study and 1% in our study). However, the resistance to TET was lower in their work (59.3%) than in this investigation (64.4%). Such differences could be attributed to particular aspects in the management of antibiotics in human and veterinary medicine.

Another investigation in Canada by Agunos et al. (2019), found resistance to be lower in *E. coli* strains from turkeys than reported in our work, specifically with regard to AMP (43% and 100%, respectively) and NAL (4% and 63%, respectively). Benklaouz et al. (2020) reported a higher frequency of resistance in *E. coli* isolates from poultry than we found our work for TET (68% and 80.6%, respectively) and SXT (26.8% and 73.7%, respectively). Differences in the management of agricultural production units, including sanitization, use of antibiotics, or production systems (but also differences in the way of sampling) could explain the discrepancies observed in several studies (Bertelloni et al. 2015; Brower et al. 2017).

On the other hand, several studies have discovered MDR *E. coli* harboring virulence factors and ARGs with clinical relevance in backyard poultry (Samanta et al. 2014). In our investigation, the bla_{TEM} gene was found in 56.3% of isolates, while *tetA* was found in 19.2%, *sulI* in 9.5%, *qnrB* in 5.5%, and *qnrA* in 9.6%. Similar frequencies of the same genes have been reported in *E. coli* strains isolated from domestic chickens in Iran (Staji et al. 2018) and from broilers with septicemia in Egypt (Ahmed et al. 2013). An important component in the fight against AMR is the detection of ARGs in animal farms. If we consider that *E. coli* is a normal commensal of gut microbiota, the possibility to generate a genetic exchange of ARGs or virulence genes between nearby animal populations, such as wild birds, increases exponentially (Lambrecht et al. 2019; Dolejska et al. 2007).

AMR to β-lactamics and tetracyclines has been reported in isolates from the gut microbiome of wild birds (Marcelino et al. 2019). In this study, the resistance to AMP of *E. coli* isolates from wild birds is lower than that reported by Shobrak and Abo-Amer (2014) in Saudi Arabia (30.7% and 75%, respectively), to NAL according to Hui et al. (2020) in Singapore (15.3% and 19.2%,

respectively), and to TET reported in Brazil by Borges et al. (2017) in *E. coli* isolates from wild birds and pigeons (19.2% and 42.8%, respectively). In contrast, the resistance to GEN was higher than reported by Rey et al. (2016) from wild birds from illegal trade markets in Rio de Janeiro (11.5% and 10.9%, respectively). However, the resistance to SXT and CIP was comparable than informed by Hui et al. (2020) in *E. coli* isolates from resident wild birds recovered from urban areas and recreational parks. There is a close link between anthropogenic activities and the acquisition of AMR in migratory birds from aquatic ecosystems.

In this study, we detected that 41.6% of *E. coli* isolates from wild birds harbored bla_{TEM}; 12.5% *sulII*, *tetA*, and *qnrA*; 8.3% *tetB*; and 4.1% both *sulI* and *qnrB*. Previous investigation, have reported the occurance of the same ARGS in *E. coli* strains of wild birds situated in rivers around areas with agricultural and livestock activities and near sewage treatment plants (Dolejska et al. 2007; Marcelino et al. 2019).

Aquatic ecosystems serve as one of the main reservoirs and trafficking routes of ARGs and virulence factors amid wildlife and anthropogenic activities, while migratory birds spread them in other ecosystems and other animal species, including humans that have contact with polluted water or through the food chain (Anand et al. 2016; Muniesa et al. 2013; Bonnedahl and Järhult 2014).

Ciénega Chimaliapan is a wetland used as a stopover for North American migratory wild birds for the fall–winter period. High pollution with organic and inorganic waste is observed, since the region is surrounded by several towns with farming and industrial activities; therefore, it is a place that supports the emergence and exchange of MDR microorganism and phages harboring ARGs (Sedeño-Díaz and López-López 2007; Hernández-Colina et al. 2018; Anand et al. 2016).

We identified 4 phages obtained in MDR *E. coli* isolates from wild migratory birds (*Anas crecca*, *Anas acuta*, *Oxyura jamaicensis*, and *Spatula discors*). Previous research in this wetland by Ramírez-Martínez et al. (2018) discovered at least six bacteriophages families (*Myoviridae*, *Spihoviridae*, *Podoviridae*, *Ackermanviridae*, *Inoviridae*, and *Microviridae*) from feces of similar migratory wild bird species (*Anas acuta*, *Mareca americana*, *Oxyura jamaicencis*, *Spatula clypeata*, *Spatula cyanoptera*, and *Spatula discors*); however, no ARGs from these phages were reported. Another study in a wetland in India illustrated a high diversity of phages from 4 families (*Microviridae*, *Myoviridae*, *Podoviridae*, and *Siphoviridae*) in the feces of waterfowl (*Anas platyrhynchos*) (Fawaz et al. 2016). These investigations confirm the ubiquity of bacteriophages in polluted aquatic environments with organic waste from livestock and agricultural activities and the relevance of these ecosystems in the transmission of ARGs (Calero-Cáseres et al. 2019).

The findings of many studies support the potential for bidirectional transmission of ARG-bearing phages between domestic and wild birds, for example, when wild birds feed and defecate on agricultural soils (Elmberg et al. 2017). In this study, the phages were able to infect 11 *E. coli* from 4 wild bird species (*Mareca strepera*, *Anas acuta*, *Spatula discors*, *Anas diazi*, *Spatula clypeata*, and *Oxyura jamaicensis*). Curiously Ross and Topp (2015) reported phages with ARGs in agricultural soils, rivers, wetlands, and other areas near urban populations that are geographic stopovers for migratory birds (Marcelino et al. 2019) and points where phages display a great diversity of ARGs (Colomer-Lluch et al. 2014*a*; Marti et al. 2014).

An indirect (wastewater treatment plant, grassland) or direct (interaction with other species) relationship among migratory wild birds harboring phages with ARGs and backyard poultry farms in rural or peri-urban areas allows the pollution of food and drink of poultry (chicken, ducks, and turkeys) with feces of wild birds, thereby creating a cycle of dissemination of these genes (Kobayashi et al. 2009; Elmberg et al. 2017; Fawaz et al. 2016).

In this work, 8 bacterial isolates from backyard poultry (4 from chicken and 4 from ducks) were infected by phages. Lima et al. (2017) discovered phages in the fecal virome from apparently healthy chickens from poultry in Brazil, while Sharma et al. (2020) detected phages in ESBL-producing *E. coli* in poultry; usually the coding genes of these proteins are linked with mobile genetic elements, including phages.

Multiple studies have shown that ARGs-bearing phages are relatively common in different environments as well human and animal microbial communities (Muniesa and Jofre 2000). Our work recorded a density of 3.8×10^5 to 6.7×10^6 PFU/mL of *E. coli* phages from wild birds. Calci et al. (1998) reported different densities of phages from domestic animals (4.4×10^3 to 1.1×10^7 PFU/mL) and waterfowl (4.4×10^3 to 5.9×10^6 PFU/mL). Furthermore, Muniesa and Jofre (1998) determined the density of environmental phages from wastewater treatment plants in Spain to be 1.8×10^4 to 2.5×10^4 PFU/mL.

The phages included in this work encompassed ARGs (*tetB*, *qnrA*, *sulII*, and bla_{TEM}) that have been described in the fecal virome of humans and animals. For example, in China, Yang et al. (2020) detected *sulI* and *sulII* genes in phages from broilers and laying hens. Other investigations reported *qnrA*, *sul1*, and bla_{TEM} genes inserted into the phages genome in rivers polluted by human and animal waste, slaughterhouse wastewater (from cattle and poultry), and wastewater treatment plants (Colomer-Lluch et al. 2011, 2014*a*, 2014*b*).

Bacteriophages could play a starring role as reservoirs of ARGs with clinical relevance. For example, Subirats et al. (2016) detected bla_{TEM} incorporated in phages from residual water samples from a hospital in Spain. Quirós

et al. (2014) reported the isolation of phages with bla_{TEM} and *qnrA* in human fecal samples. Finally, the *sulI*, bla_{TEM}, and *qnrA* genes harbored by phages of 3 different families could be detected before and after a regimen with ciprofloxacin in fecal human samples (Fernández-Orth et al. 2019).

Several investigations mention that animals and humans apparently healthy (with or without antibiotic treatments) excrete phages that carry numerous ARGs and these can be disseminated in aquatic ecosystems (rivers, swamps, and wastewater treatment plants) where wild birds can attain them and spread them constantly during migration (Quirós et al. 2014; Fernández-Orth et al. 2019; Elmberg et al. 2017; Calero-Cáseres et al. 2019).

Aquatic ecosystems can be strategic sites where transduction and lysogenic conversion take place and contribute to the emergence of new MDR strains. In this investigation, phage T22 transduced the *tetB* and bla_{TEM} genes to 4 recipient isolates (2 from backyard poultry and 2 wild birds), and T1 transduced the *tetB* gene to 1 *E. coli* isolate from backyard poultry and 3 isolates from wild birds. All recipient isolates showed lysogenic conversion. Shousha et al. (2015) reported the lysogenic conversion of 5 *E. coli* reference strains employing phages collected from chicken meat carrying *tetA* and bla_{TEM} genes. Another example is the transduction of a pSTS7-like resistance plasmid that confers resistance against tetracyclines and aminoglycosides to *Staphylococcus aureus* and *Staphylococcus sciuri* through phage ϕ879 obtained from *S. sciuri* (Zeman et al. 2017).

Four *E. coli* isolates (2 from backyard poultry and 2 from wild birds) showed lysogenic conversion when *sulII* and *qnrA* genes were transduced by phage T21. Muniesa et al. (2011) pointed out that successful environmental transduction depends on adequate concentrations of phage and bacteria, an optimal bacterial physiological state, and suitable environmental conditions. Additionally, Colavecchio et al. (2017) suggest that some phages are more prone to acquire and disseminate certain ARGs in different ecosystems. We also found that 7 *E. coli* isolates (3 from backyard poultry and 4 wild birds) were lysogenized by phage T11 that harbored the *qnrA* gene. The incorporation of ARGs and virulence factors to bacterial strains through the transduction phenomenon provide conditions for the emergence of new pathogen strains (Eichhorn et al. 2018).

The bacteriophage's proficiency to transport and spread ARGs and virulence factors in different environments is an epidemiological risk for people that live daily in animal production units, especially those who live in rural and peri-urban areas. For example, Eichhorn et al. (2018) carried out an experiment in which *stx* genes were successfully lysogenized to 3 atypical enteropathogenic *E. coli* (murine, human, and bovine) with zoonotic potential using the phage ϕ3538 Δstx$_2$:cat that was present in isolates of enterohaemorrhagic *E. coli*. Other investigation demonstrated that commensal *E. coli* from children and adults were lysogenized by phage Φ734, which harbors the *stx2* gene (Iversen et al. 2015). It is evident that phages have a significant performance in bacterial diversity, ecology, and evolution.

Finally, this work is the first to demonstrate the in vitro transduction phenomenon of ARGs between phages from migratory wild-birds and poultry *E. coli* isolates and show the need to include and broaden studies in backyard poultry farms and wildlife as well as monitor the dissemination route of phages with ARGs related to AMR.

Acknowledgements

This work was funded by Universidad Autónoma del Estado de México, Project 5000/2020CBI.

References

Aguilar-Montes de Oca, S., Talavera-Rojas, M., Soriano-Vargas, E., Barba-León, J., and Vazquez-Navarrete, J. 2015. Determination of extended spectrum β-lactamases/AmpC β-lactamases and plasmid-mediated quinolone resistance in *Escherichia coli* isolates obtained from bovine carcasses in Mexico. Trop. Anim. Health Prod. **47**: 975–981. doi:10.1007/s11250-015-0818-3. PMID: 25894820.

Agunos, A., Gow, S.P., Léger, D.F., Carson, C.A., Deckert, A.E., Bosman, A.L., et al. 2019. Antimicrobial use and antimicrobial resistance indicators — integration of farm-level surveillance data from broiler chickens and turkeys in British Columbia, Canada. Front. Vet. Sci. **6**: 131. doi:10.3389/fvets.2019.00131. PMID:31131285.

Ahmed, A.M., Shimamoto, T., and Shimamoto, T. 2013. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int. J. Med. Microbiol. **303**(8): 475–483. doi:10.1016/j.ijmm.2013.06.009. PMID:23891276.

Alcalá, L., Alonso, C.A., Simón, C., González-Esteban, C., Orós, J., Rezusta, A., et al. 2016. Wild birds, frequent carriers of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* of CTX-M and SHV-12 types. Microb. Ecol. **72**(4): 861–869. doi:10.1007/s00248-015-0718-0. PMID:26687342.

Amin, M.B., Sraboni, A.S., Hossain, M.I., Roy, S., Mozmader, T.A.U., Unicomb, L., et al. 2020. Occurrence and genetic characteristics of *mcr*-1-positive colistin-resistant *E. coli* from poultry environments in Bangladesh. J. Glob. Antimicrob. Resist. **22**: 546–552. doi:10.1016/j.jgar.2020.03.028. PMID:32344122.

Anand, T., Bera, B.C., Vaid, R.K., Barua, S., Riyesh, T., Virmani, N., et al. 2016. Abundance of antibiotic resistance genes in environmental bacteriophages. J. Gen. Virol. **97**(12): 3458–3466. doi:10.1099/jgv.0.000639. PMID:27902329.

Armas-Freire, P., Trueba, G., Proaño-Bolaños, C., Levy, K., Zhang, L., Marrs, C.F., et al. 2015. Unexpected distribution of the fluoroquinolone-resistance gene *qnrB* in *Escherichia coli* isolates from different human and pultry origins in Ecuador. Int. Microbiol. **18**(2): 85–90. doi:10.2436/20.1501.01.237. PMID:26496615.

Benklaouz, M.B., Aggad, H., and Benameur, Q. 2020. Resistance to multiple first-line antibiotics among *Escherichia coli* from poultry in Western Algeria. Vet. World, **13**(2): 290–295. doi:10.14202/vetworld.2020.290-295. PMID:32255971.

Bertelloni, F., Salvadori, C., Moni, A., Cerri, D., Mani, P., and Ebani, E.V. 2015. Antimicrobial resistance in *Enterococcus* spp. isolated from laying hens of backyard poultry floks.

Ann. Agric. Environ. Med. **22**(4): 665–669. doi:10.5604/12321966.1185771. PMID:26706973.

Bonnedahl, J., and Järhult, J.D. 2014. Antibiotic resistance in wild birds. Ups. J. Med. Sci. **119**(2): 113–116. doi:10.3109/03009734.2014.905663. PMID:24697355.

Borges, C.A., Cardozo, M.V., Beraldo, L.G., Oliveira, E.S., Maluta, R.P., Barboza, K.B., et al. 2017. Wild birds and urban pigeons as reservoirs for diarrheagenic *Escherichia coli* with zoonotic potential. J. Microbiol. **55**(5): 344–348. doi:10.1007/s12275-017-6523-3. PMID:28281197.

Brower, C.H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S.J., et al. 2017. The prevalecte of extended-spectrum β-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. Environ. Health Perspect. **125**(7): 077015. doi:10.1289/EHP292. PMID:28749780.

Calci, K.R., Burkhardt, W., Watkins, W.D., and Rippey, S.R. 1998. Occurrence of male-specific bacteriophage in feral and domestic animal waste, human feces, and human-associated wastewaters. Appl. Environ. Microbiol. **64**(12): 5027–5029. doi:10.1128/AEM.64.12.5027-5029.1998. PMID:9835602.

Calero-Cáseres, W., Ye, M., and Balcázar, J.L. 2019. Bacteriophages as environmental reservoirs of antibiotic resistance. Trends Microbiol. **27**(7): 570–577. doi:10.1016/j.tim.2019.02.008. PMID:30905524.

CEDRSSA (Centro de Estudios por el Desarrollo Rural Sustentable y la Soberanía Alimentaria). 2019. La importancia de la Industria Avícola en México. CEDRSSA. Palacio Legislativo de San Lazaro, Mexico City.

CLSI (Clinical and Laboratory Standard Institute). 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests - 19th edition. Approved Standard M02-A11. CLSI, Wayne, Penn., USA.

CLSI (Clinical and Laboratory Standard Institute). 2015. Performance Standards for Antimicrobial Susceptibility Testing, 25nd informational supplement. M100-S25. CLSI, Wayne, Penn., USA.

Colavecchio, A., Cadieux, B., Lo, A., and Goodridge, L.D. 2017. Bacteriophages contribute to the spread of antibiotic resistance genes among foodborne pathogens of the *Enterobacteriaceae* family–a review. Front. Microbiol. **8**: 1108. doi:10.3389/fmicb.2017.01108. PMID:28676794.

Colomer-Lluch, M., Jofre, J., and Muniesa, M. 2011. Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. PLoS One, **6**(3): e17549. doi:10.1371/journal.pone.0017549. PMID:21390233.

Colomer-Lluch, M., Jofre, J., and Muniesa, M. 2014*a*. Quinolone resistance genes (*qnrA* and *qnrS*) in bacteriophage particles from wastewater sample and the effect of inducing agents on packaged antibiotic resistance genes. J. Antimicrob. Chemother. **69**(5): 1265–1274. doi:10.1093/jac/dkt528. PMID:24458509.

Colomer-Lluch, M., Calero-Cáceres, W., Jebri, S., Hmaied, F., Muniesa, M., and Jofre, J. 2014*b*. Antibiotic resistance genes in bacterial and bacteriophage fractions of Tunisian and Spanish wastewater as markers to compare the antibiotic resistance patterns in each population. Environ. Int. **73**: 167–175. doi:10.1016/j.envint.2014.07.003. PMID:25127043.

Dallenne, C., da Costa, A., Decré, V., Favier, C., and Arlet, G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. **65**(3): 490–495. doi:10.1093/jac/dkp498. PMID:20071363.

Dolejska, M., Cizek, A., and Literak, I. 2007. High prevalence of antimicrobial – resistant genes and integrons in *Escherichia coli* isolates from Black – headed Gulls in the Czech Republic. J. Appl. Microbiol. **103**(1): 11–19. doi:10.1111/j.1365-2672.2006.03241.x. PMID:17584448.

Eichhorn, I., Heidemanns, K., Ulrich, R.G., Schmidt, H., Semmler, T., Fruth, A., et al. 2018. Lysogenic conversion of atypical enteropathogenic *Escherichia coli* (aEPEC) from human, murine, and bovine origin with bacteriophage ϕ3538 Δstx2::cat proves their enterohemorrhagic *E. coli* (EHEC) progeny. Int. J. Med. Microbiol. **308**(7): 890–898. doi:10.1016/j.ijmm.2018.06.005. PMID:29937391.

Elmberg, J., Berg, C., Lerner, H., Waldenström, J., and Hessel, R. 2017. Potential disease transmission from wild geese and swans to livestock, poultry and humans: a review of the scientific literature from a One Health perspective. Infect. Ecol. Epidemiol. **7**(1): 1300450. doi:10.1080/20008686.2017.1300450. PMID:28567210.

Fawaz, M., Vijayakumar, P., Mishra, A., Gandhale, P.N., Dutta, R., Kamble, N.M., et al. 2016. Duck gut viral metagenome analysis captures snapshot of viral diversity. Gut Pathog. **8**: 30. doi:10.1186/s13099-016-0113-5. PMID:27284287.

Fernández-Orth, D., Miró, E., Brown-Jaque, M., Rodríguez-Rubio, L., Espinal, P., Rodríguez-Navarro, J., et al. 2019. Faecal phageome of healthy individuals: presence of antibiotic resistance genes and variations caused by criprofloxacin treatment. J. Antimicrob. Chemother. **74**(4): 854–864. doi:10.1093/jac/dky540. PMID:30649322.

Fortier, L.C., and Moineau, S. 2009. Phage production and maintenance of stocks, including expected stock lifetimes. *In* Bacteriophages: methods and protocols. *Edited by* M.R.J. Clokie and A.M. Kropinski. Springer Science + Business, Volumen 501 of Humana Press, pp. 203–219.

García-Aljaro, C., Muniesa, M., Jofre, J., and Blanch, A.R. 2006. Newly identified bacteriophages carrying the stx_{2g} Shiga toxin gene isolated from *Escherichia coli* strains in polluted waters. FEMS Microbiol. Lett. **258**(1): 127–135. doi:10.1111/j.1574-6968.2006.00213.x. PMID:16630267.

Hedayatianfard, K., Akhlaghi, M., and Sharifiyazdi, H. 2014. Detection of tetracycline resistance genes in bacteria isolated from fish farms using polymerase chain reaction. Vet. Res. Forum, **5**(4): 269–275 PMID:25610578.

Heijnen, L., and Medema, G. 2006. Quantitative detection of *E. coli*, *E. coli* O157 and other shiga toxin producing *E. coli* in water samples using a culture method combined with real-time PCR. J. Water Health, **4**(4): 487–498. doi:10.2166/wh.2006.0032. PMID:17176819.

Hernández-Colina, A., Yadeun, M., and García-Espinosa, G. 2018. Comunidad de aves acuáticas de un humedal protegido en el Estado de México, México. Huitzil, **19**(1): 85–95. doi:10.28947/hrmo.2018.19.1.310.

Hui, O.K., Ching, K.W., Yi, Q.J., Xi, L.Z., Arivalan, S., Humaidi, M., et al. 2020. Occurrence and antimicrobial resistance traits of *Escherichia coli* from wild birds and rodents in Singapore. Int. J. Environ. Res. Public Health, **17**(15): 5606. doi:10.3390/ijerph17155606. PMID:32756497.

Iversen, H., L' Abée-Lund, T.M., Aspholm, M., Arnesen, L.P.S., and Lindbäck, T. 2015. Commensal *E. coli* Stx2 lysogens produce high levels of phages after spontaneous prophage induction. Front. Cell. Infect. Microbiol. **5**: 5. doi:10.3389/fcimb.2015.00005. PMID:25692100.

Kerrn, M.B., Klemmensen, T., Frimodt-Møller, N., and Espersen, F. 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**(4): 513–516. doi:10.1093/jac/dkf164. PMID:12356795.

Kissinga, H.D., Mwombeki, F., Said, K., Katakweba, A.A.S., Nonga, H.E., and Muhairwa, A.P. 2018. Antibiotic susceptibilities of indicator bacteria *Escherichia coli* and *Enterococci* spp. isolated from ducks in Morogoro Municipality. BMC Res Notes, **11**(1): 87. doi:10.1186/s13104-018-3201-4. PMID:29386067.

Kobayashi, H., Kanazaki, M., Hata, E., and Kubo, M. 2009. Prevalence and characteristics of *eae*- and *stx*-positive strains of *Escherichia coli* from wild birds in the immediate environment of Tokyo Bay. Appl. Environ. Microbiol. **75**(1): 292–295. doi:10.1128/AEM.01534-08. PMID:18997019.

Koga, V.L., Scandorieiro, S., Vespero, E.C., Oba, A., de Brito, B.G., de Brito, K.C.T., et al. 2015. Comparison of antibiotic resistance and virulence factor among *Escherichia coli* isolated from conventional and free-range poultry. BioMed Res. Int. **2015**: 618752. doi:10.1155/2015/618752. PMID:26579536.

Kraychete, G.B., Botelho, L.A., Campana, E.H., Picão, R.C., and Bonelli, R.R. 2016. Updated multiplex PCR for detection of all six plasmid-mediated *qnr* gene families. Antimicrob. Agents Chemother. **60**(12): 7524–7526. doi:10.1128/AAC.01447-16. PMID:27736767.

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E., and Johnson, R.P. 2009. Enumeration of bacteriophages by double agar overlay plaque assay. *In* Bacteriophages: Methods and Protocols. *Edited by* M.R.J. Clokie and A.M. Kropinski. Springer Science + Business, Volume 501 of Humana Press. pp. 69–76.

Lambrecht, E., Van, C.E., Van, M.E., Boon, N., Heyndrickx, M., and Van de Wiele, T. 2019. Commensal *E. coli* rapidly transfer antibiotic resistance genes to human intestinal microbiota in the Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME). Int. J. Food Microbiol. **311**: 108357. doi:10.1016/j.ijfoodmicro.2019.108357. PMID:31536878.

Lima, D.A., Cibulski, S.P., Finkler, F., Teixeira, T.F., Varela, A.P.M., Cerva, C., et al. 2017. Fecal virome of healthy chickens reveals a large diversity of the eukaryote viral community, including novel circular ssDNA viruses. J. Gen. Virol. **98**(4): 690–703. doi:10.1099/jgv.0.000711. PMID:28100302.

Marcelino, V.R., Wille, M., Hurt, A.C., González-Acuña, D., Klaassen, M., Schlub, T.E., et al. 2019. Meta-transcriptomics reveals a diverse antibiotic resistance gene pool in avian microbiomes. BMC Biol. **17**(1): 31. doi:10.1186/s12915-019-0649-1. PMID:30961590.

Marti, E., Variatza, E., and Balcázar, J.L. 2014. Bacteriophages as a reservoir of extended-spectrum β-lactamase and fluoroquinolone resistance genes in the environment. Clin. Microbiol. Infect. **20**(7): 456–459. doi:10.1111/1469-0691.12446. PMID:24552593.

Muniesa, M., and Jofre, J. 1998. Abundance in sewage of bacteriophages that infect *Escherichia coli* O157:H7 and that carry the shiga toxin 2 gene. Appl. Environ. Microbiol. **64**(7): 2443–2448. doi:10.1128/AEM.64.7.2443-2448.1998. PMID:9647813.

Muniesa, M., and Jofre, J. 2000. Occurrence of phages infecting *Escherichia coli* O157:H7 carrying the Stx 2 in sewage from different countries. FEMS Microbiol. Lett. **183**(1): 197–200. doi:10.1111/j.1574-6968.2000.tb08957.x. PMID:10650226.

Muniesa, M., Imamovic, L., and Jofre, J. 2011. Bacteriophages and genetic mobilization in sewage and faecally polluted environments. Microb. Biotechnol. **4**(6): 725–734. doi:10.1111/j.1751-7915.2011.00264.x. PMID:21535427.

Muniesa, M., Colomer-Luch, M., and Jofre, J. 2013. Could bacteriophages transfer antibiotic resistance genes from environmental bacteria to human-body associated bacterial populations?. Mob. Genet. Elements, **3**(4): e25847. doi:10.4161/mge.25847. PMID:24195016.

Okpara, E.O., Ojo, O.E., Awoyomi, O.J., Dipeolu, M.A., Oyekunle, M.A., and Schwarz, S. 2018. Antimicrobial usage and presence of extended-spectrum β-lactamase-producing Enterobacteriaceae in animal-rearing households of selected rural and peri-urban communities. Vet. Microbiol. **218**: 31–39. doi:10.1016/j.vetmic.2018.03.013. PMID:29685218.

Parker, D., Sniatynski, M.K., Mandrusiak, D., and Rubin, J.E. 2016. Extended-spectrum β-lactamase producing *Escherichia coli* isolated from wild birds in Saskatoon, Canada. Lett. Appl. Microbiol. **63**(1): 11–15. doi:10.1111/lam.12589. PMID:27214496.

Quirós, P., Colomer-Lluch, M., Martínez-Castillo, A., Miró, E., Argente, M., Jofre, J., et al. 2014. Antibiotic resistance genes in the bacteriophage DNA fraction of human fecal samples. Antimicrob. Agents Chemother. **58**(1): 606–609. doi:10.1128/AAC.01684-13. PMID:24165177.

Ramírez-Martínez, L., Loza-Rubio, E., Mosqueda, J., González-Garay, M.L., and García-Espinoza, G. 2018. Fecal virome composition of migratory wild duck species. PLoS One, **13**(11): e0206970. doi:10.1371/journal.pone.0206970. PMID:30462678.

Rey, M.C.A., Pereira, I.A., Falavina, R.E.M., Prazeres, R.D., and Siciliano, S. 2015. Frequency of zoonotic bacteria among illegally traded wild birds in Rio de Janeiro. Br. J. Microbiol. **47**(4): 882–888. doi:10.1016/j.bjm.2016.07.012. PMID:27528081.

Ross, J., and Topp, E. 2015. Abundance of antibiotic resistance genes in bacteriophage following soil fertilization with dairy manure or municipal biosolids, and evidence for potential transduction. Appl Environ. Microbiol. **81**(22): 7905–7913. doi:10.1128/AEM.02363-15. PMID:26341211.

Samanta, I., Joardar, S.N., Das, P.K., Das, P., Sar, T.K., Dutta, T.K., et al. 2014. Virulence repertoire, characterization, and antibiotic resistance pattern analysis of *Escherichia coli* isolated from backyard layers and their environmental in India. Avian Dis. **58**(1): 39–45. doi:10.1637/10586-052913-Reg.1.

Sedeño-Díaz, J., and López-López, E. 2007. Water quality in the Río Lerma, Lerma: an overview of the last quarter of the twentieth century. Water Resour. Manage. **21**(10): 1797–1812. doi:10.1007/s11269-006-9128-x.

Sekulović, O., and Fortier, L.C. 2016. Characterization of functional prophages in *Clostridium difficile*. *In* Clostridium difficile: Methods and Protocols, Volume 1: Isolation, Characterization and Interactions. *Edited by* A.P. Roberts and P. Mullany. Springer Science + Business, New York. pp. 143–165.

Sharma, P., Gupta, S.K., Adenipekun, E.O., Barret, J.B., Hiott, L.M., Woodley, T.A., et al. 2020. Genome analysis of multidrug-resistant *Escherichia coli* isolated from poultry in Nigeria. Foodborne Pathog. Dis. **17**(1): 1–7. doi:10.1089/fpd.2019. PMID:31509034.

Shobrak, M.Y., and Abo-Amer, A.E. 2014. Role of wild birds as carriers of multi-drug resistant *Escherichia coli* and *Escherichia vulneris*. Braz. J. Microbiol. **45**(4): 1199–1209. doi:10.1590/s1517-83822014000400010. PMID:25763023.

Shousha, A., Awaiwanont, N., Sofka, D., Smulders, F.J.M., Paulsen, P., Szostak, M.P., et al. 2015. Bacteriophages isolated from chicken meat and the horizontal transfer of antimicrobial resistance genes. Appl. Environ. Microbiol. **81**(14): 4600–4606. doi:10.1128/AEM.00872-15. PMID:25934615.

Staji, H., Tonelli, A., Zahraei, S.T., Mahdavi, A., Shahroozian, E., Salimi, B.M.R., et al. 2018. Distribution of antibiotic resistance genes among the phylogroups of *Escherichia coli* in diarrheic calves and chickens affected by colibacillosis in Tehran. Arch. Razi Inst. **73**(2): 131–137. doi:10.22092/ari.2018.116502. PMID:30242804.

Subirats, J., Sànchez-Melsió, A., Borrego, C.M., Balcázar, J.L., and Simonet, P. 2016. Metagenomic analysis reveals that bacteriophages are reservoirs of antibiotic resistance genes. Int. J. Antimicrob. Agents, **48**(2): 163–167. doi:10.1016/j.ijantimicag.2016.04.028. PMID:27312355.

van den Bogaard, A.E., London, N., Driessen, C., and Stobberinhg, E.E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. J. Antimicrob. Chemother. **47**(6): 763–771. doi:10.1093/jac/47.6.763. PMID:11389108.

van Hoek, A.H.A.M., Stalenhoef, J.E., van Duijkeren, E., and Franz, E. 2016. Comparative virulotyping of extended-spectrum cephalosporin-resistant *E. coli* isolated from broilers, humans on broiler farms and in the general population

and UTI patients. Vet. Microbiol. **194**: 55–61. doi:10.1016/j.vetmic.2016.04.008. PMID:27106522.

Yang, Y., Xie, X., Tang, M., Liu, J., Tuo, H., Gu, J., et al. 2020. Exploring the profile of antimicrobial resistance genes harboring by bacteriophage in chicken feces. Sci. Total Environ. **700**: 134446. doi:10.1016/j.scitotenv.2019.134446. PMID:31648121.

Zeman, M., Mašlaňová, I., Indráková, A., Šiborová, M., Mikulášek, K., Bendíčková, K., et al. 2017. *Staphylococcus sciuri* bacteriophages double-convert for staphylokinase and phospholipase, mediate interspecies plasmid transduction, and package *mecA* gene. Sci. Rep. **7**: 46319. doi:10.1038/srep46319. PMID:28406168.