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Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* in Enteric Microflora of Wild Ducks

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ABSTRACT: We tested 772 cloacal swabs from wild ducks to estimate the prevalence of enteric bacteria resistant to extended-spectrum cephalosporins (ESC). We found a low prevalence of the important ESC resistance genotypes, bla_{CMY} (5.7%) and $bla_{\text{CTX-M}}$ (0.3%). This suggests a minor role for wild ducks in the movement of resistant bacteria in the environment.

Antimicrobial resistance is of critical importance, with significant negative consequences for health care (Razazi et al. 2012). Resistance to extended-spectrum cephalosporins (ESC) has been identified as a serious threat to human health (Centers for Disease Control 2013). Livestock have been implicated in the transmission of ESC-resistant bacteria to humans via food-borne transmission (Mora et al. 2010; López-Cerero et al. 2011). Livestock and food products can harbor bacteria with ESC resistance, primarily from plasmid-mediated β-lactamase genes conferring resistance to ESC, including the AmpC bla_{CMY} and the extended spectrum β-lactamase $bla_{\text{CTX-M}}$ (Mollenkopf et al. 2014; Davis et al. 2015).

Wild ducks have been associated with the transmission of livestock pathogens and Shigatoxigenic *Escherichia coli* (Ewers et al. 2009; Kim et al. 2009). In Europe and Asia, wild waterfowl have been shown to harbor ESC-resistant bacteria in their gastrointestinal (GI) flora (Guenther et al. 2010; Veldman et al. 2013; Mohsin et al. 2016). However, little is known of their role in the transmission or movement of ESC-resistant bacteria in the US. Wild ducks have direct contact with water ecosystems, potentially contaminating many environments and populations. We investigated wild ducks as a reservoir for ESC-resistant *Enterobacteriaceae*. Specifically, we determined the prevalence of the AmpC (bla_{CMY}) and extended-spectrum β-lactamase ($bla_{\text{CTX-M}}$) resistance genes, which are plasmid-mediated genes that can readily move between bacterial species via horizontal gene transfer (Winokur et al. 2000).

Ducks were caught during routine waterfowl surveillance in conjunction with the Ohio Department of Natural Resources throughout Ohio and in conjunction with Winous Point Marsh Conservancy along the shoreline of Lake Erie in Ohio from July 2014–April 2015. Cloacal swabs were obtained with sterile Stuart's liquid media transport swabs (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Birds were banded with an aluminum band and released. Additionally, cloacal swabs were obtained from hunter-harvested ducks throughout the 2014–15 hunting season during routine surveillance by the Ohio Department of Natural Resources, Winous Point Marsh Conservancy, and by a field technician along the Mississippi flyway in Illinois, Wisconsin, Mississippi, Arkansas, Iowa, and Missouri.

Cloacal swabs were appropriately stored for up to 2 wk and transported to the laboratory at ambient temperature. Swabs were incubated overnight in 10 mL of buffered peptone water (Becton, Dickinson and Company); a 1-mL aliquot was then incubated overnight in 9 mL of MacConkey broth (Becton, Dickinson and Company) modified with 2 μg/mL of cefotaxime (TCI America, Portland, Oregon, USA). MacConkey broth was inoculated onto MacConkey agar (EMD Millipore, Darmstadt, Germany) modified with 8 μg/mL cefoxitin (CHEM-IMPEX INT'L, Wood Dale, Illinois, USA), 4 μg/mL cefepime (CHEM-IMPEX), or 1 μg/mL meropenem (US Pharmacopeial

TABLE 1. Number (prevalence) of wild ducks with enteric bacteria harboring bla_{CMY} and $bla_{CTX\text{-}M}$ resistance genes sampled from July 2014–April 2015 along the central flyway in the USA, comparing hunter-harvested and live-caught ducks.

	Sources of ducks[a]							
	Overall			Hunter harvested			Live caught	
Sampling Location	Total	bla_{CMY}	$bla_{CTX\text{-}M}$	Total	bla_{CMY}	$bla_{CTX\text{-}M}$	bla_{CMY}	$bla_{CTX\text{-}M}$
Ohio	662	37	2	384	9	2	28	0
Winous Point Marsh Conservancy	618	26	2	378	7	2	19	0
Pickeral Creek	38	9	0	—	—	—	9	0
Big Island	6	2	0	6	2	0	—	—
Illinois	38	4	0	38	4	0	—	—
Big Slough	16	4	0	16	4	0	—	—
Carlyle Lake	10	0	0	10	0	0	—	—
Shelbyville Lake	6	0	0	6	0	0	—	—
Rend Lake	6	0	0	6	0	0	—	—
Wisconsin	22	2	0	22	2	0	—	—
Horicon	12	1	0	12	1	0	—	—
Grand River	10	1	0	10	1	0	—	—
Arkansas	16	0	0	16	0	0	—	—
5 Oaks	16	0	0	16	0	0	—	—
Mississippi	15	0	0	15	0	0	—	—
Howard Miller	13	0	0	13	0	0	—	—
Mahanna	2	0	0	2	0	0	—	—
Iowa	11	0	0	11	0	0	—	—
Lansing	11	0	0	11	0	0	—	—
Missouri	8	1	0	8	1	0	—	—
Otter Slough	8	1	0	8	1	0	—	—
Totals, No. (%)	772	44 (5.7)	2 (0.3)	494	16 (3.2)	2 (0.4)	28 (10.1)	0

[a] — = no samples collected.

Convention, Rockville, Maryland, USA) to identify bla_{CMY}, $bla_{CTX\text{-}M}$, and carbapenem-resistant phenotypes, respectively, and incubated overnight. Up to three lactose fermenting, indole-positive isolates representing distinct morphologies were presumed to be *E. coli* and were selected for further characterization from each selective agar. For *Salmonella* screening, a 100-µL aliquot of buffered peptone water was incubated overnight in 10 mL of Rappaport Vassiliadis broth (Becton, Dickinson and Company), then inoculated onto XLT4 agar (Becton, Dickinson and Company) and incubated overnight. A single, characteristic black colony was selected and grown on nonselective MacConkey agar. A nonlactose-fermenting colony was selected for *Salmonella* polyvalent O antisera agglutination and inoculated onto a Triple Sugar Iron agar (Becton, Dickinson and Company).

Identification of bla_{CMY} and $bla_{CTX\text{-}M}$ was performed using standard PCR techniques with primers previously reported (Mollenkopf et al. 2012). We bidirectionally Sanger-sequenced $bla_{CTX\text{-}M}$ genes using the corresponding PCR amplification primers and analyzed them using BLAST (NCBI 2016).

A total of 772 wild ducks were sampled in July 2014–April 2015; 278 were live caught, and 494 were hunter harvested. Samples were collected from 14 different sampling sites in seven states; none of which were in close proximity to any large metropolitan areas (Table 1). Samples were collected from ducks representing 15 different Anseriformes species.

TABLE 2. Number (prevalence) of wild ducks with enteric bacteria harboring bla_{CMY} and $bla_{CTX\text{-}M}$ resistance genes sampled from July 2014–April 2015 along the central flyway in the USA, comparing ducks sampled in the warm and cold seasons.

Sampling locations	Seasons ducks sampled[a]						Distances from closest large Metropolitan area (km)[b]
	Warm season (April–September)			Cold season (October–March)			
	Total	bla_{CMY}	$bla_{CTX\text{-}M}$	Total	bla_{CMY}	$bla_{CTX\text{-}M}$	
Ohio	326	33	0	330	4	2	
Winous Point Marsh Conservancy	282	22	0	330	4	2	72 from Toledo
Pickeral Creek	38	9	0	—	—	—	74 from Toledo
Big Island	6	2	0	—	—	—	87 from Columbus
Illinois	—	—	—	38	4	0	
Big Slough	—	—	—	16	4	0	108 from Rockford
Carlyle Lake	—	—	—	10	0	0	93 from St. Louis
Shelbyville Lake	—	—	—	6	0	0	100 from Springfield
Rend Lake	—	—	—	6	0	0	132 from St. Louis
Wisconsin	—	—	—	22	2	0	
Horicon	—	—	—	12	1	0	93 from Milwaukee
Grand River	—	—	—	10	1	0	87 from Madison
Arkansas	—	—	—	16	0	0	
5 Oaks	—	—	—	16	0	0	101 from Little Rock
Mississippi	—	—	—	15	0	0	
Howard Miller	—	—	—	13	0	0	143 from Jackson
Mahanna	—	—	—	2	0	0	97 from Jackson
Iowa	11	0	0	—	—	—	
Lansing	11	0	0	—	—	—	159 from Rochester
Missouri	—	—	—	8	1	0	
Otter Slough	—	—	—	8	1	0	219 from Memphis
Totals, No. (%)	335	33 (9.9)	0	437	11 (2.5)	2 (0.5)	

[a] — = no samples collected.

[b] Metropolitan areas with a population of >100,000 people were considered large Metropolitan areas.

Among hunter-harvested ducks, 384 were from Ohio, 38 from Illinois, 22 from Wisconsin, 15 from Mississippi, 16 from Arkansas, 11 from Iowa, and eight from Missouri (Table 1). The most common hunter-harvested ducks sampled were Mallards (*Anas platyrhynchos*), Green-winged Teal (*Anas carolinensis*), and Blue-winged Teal (*Anas discors*). Among live-caught ducks, all sampled in Ohio, the most common ducks sampled were Mallards, Wood Ducks (*Aix sponsa*), and Redheads (*Aythya americana*). There were 44 ducks that harbored bla_{CMY} in their GI flora, representing 5.7% of the population: 10.1% of the live-caught ducks and 3.2% of the hunter-harvested ducks (Table 1). These 44 bla_{CMY} harboring ducks were sampled from four states; 37 from Ohio, four from Illinois, two from Wisconsin, and one from Missouri; 9.9% were sampled in the warm season (April–September), and 2.5% were from the cold season (October–March; Tables 1, 2). Among the 44 ducks that harbored bla_{CMY}, 23 were Mallards, nine were Wood Ducks, seven were Blue-wing Teal, two each were Gadwall (*Anas strepera*) and Green-wing Teal, and one was a Northern Pintail (*Anas acuta*). Only two ducks, a Mallard and a Northern Shoveler (*Anas clypeata*), harbored $bla_{CTX\text{-}M}$ in their GI flora, representing 0.3% of the population; both were hunter-harvested ducks from Ohio in the cold season (Tables 1, 2). Both $bla_{CTX\text{-}M}$ genes were identified as $bla_{CTX\text{-}M\text{-}15}$, the most com-

mon extended-spectrum β-lactamase found in humans, and occasionally reported in food-producing animal populations (Watson et al. 2012; Chen et al. 2014). Six wild ducks, all of which were hunter harvested from Ohio, were culture positive for *Salmonella* spp., representing 0.8% of the sampled population; none of which were resistant to ESC.

Our results indicated that wild ducks can harbor clinically relevant antimicrobial-resistance genes in their GI flora. Many ducks are migratory and can travel great distances, leading to the potential for regional or international movement of these resistance genes, as documented with other pathogens (Gaidet et al. 2008). Although ducks have limited direct contact with livestock species, they have been reported to transmit infectious pathogens to food animal populations (Kim et al. 2009), where they can be introduced into the food supply. Given the mobility of ducks, resistance genes can be disseminated to multiple waterways with the potential for direct human contact via recreational water use or to livestock facilities. This type of community-acquired infection can be important because 78% of Canadian extended-spectrum β-lactamase-producing *E. coli* bloodstream infections reportedly originated in the community (Peirano et al. 2012). Wildlife, especially birds, which can have a role in community exposure by contaminating aquatic ecosystems, represents a potential source of transmission to livestock species and is a direct food source when hunter harvested. Food animal populations have much higher levels of bla_{CMY} and $bla_{\text{CTX-M}}$, most likely because of the high level of antimicrobial selection pressure in those populations. In the US, 70% of dairies have been shown to have >80% of their cows harboring bla_{CMY}, and 76% of dairies have >40% of their cows harboring $bla_{\text{CTX-M}}$ (Davis et al. 2015). Hospitalized human populations, however, have a similar prevalence of bla_{CMY} in their enteric flora (10%) and a higher prevalence of $bla_{\text{CTX-M}}$ (3%; Landers et al. 2016). However, because of the lower observed prevalence of ESC-resistance genes in this study, the role of wild ducks in the dissemination of clinically relevant, antibiotic-resistant bacteria and resistance genes may be limited, relative to other reservoirs of antimicrobial resistance.

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LITERATURE CITED

Centers for Disease Control and Prevention. 2013. *Antibiotic resistance threats in the United States*. http://www.cdc.gov/drugresistance/threat-report-2013. Accessed September 2016.

Chen LF, Freeman JT, Nicholson B, Keiger A, Lancaster S, Joyce M, Woods CW, Cook E, Adcock L, Louis S, et al. 2014. Widespread dissemination of CTX-M-15 genotype extended-spectrum-β-lactamase-producing *Enterobacteriaceae* among patients presenting to community hospitals in the southeastern United States. *Antimicrob Agents Chemother* 58:1200–1202.

Davis MA, Sischo WM, Jones LP, Moore DA, Ahmed S, Short DM, Besser TE. 2015. Recent emergence of *Escherichia coli* with cephalosporin resistance conferred by $bla_{\text{CTX-M}}$ on Washington State dairy farms. *Appl Environ Microbiol* 81:4403–4410.

Ewers C, Guenther S, Wieler LH, Schierack P. 2009. Mallard ducks—A waterfowl species with high risk of distributing *Escherichia coli* pathogenic for humans. *Env Microbiol Rep* 1:510–517.

Gaidet N, Newman SH, Hagemeijer W, Dodman T, Cappelle J, Hammoumi S, De Simone L, Takekawa JY. 2008. Duck migration and past influenza A (H5N1) outbreak areas. *Emerg Infect Dis* 14:1164–1166.

Guenther S, Grobbel M, Beutlich J, Bethe A, Friedrich ND, Goedecke A, Lübke-Becker A, Guerra B, Wieler LH, Ewers C. 2010. CTX-M-15-type extended-spectrum beta-lactamases-producing *Escherichia coli* from wild birds in Germany. *Env Microbiol Rep* 2: 641–645.

Kim JK, Negovetich NJ, Forrest HL, Webster RG. 2009. Ducks: The "Trojan horses" of H5N1 influenza. *Influenza Other Respir Viruses* 3:121–128.

Landers TF, Mollenkopf DF, Faubel RL, Dent A, Pancholi P, Daniels JB, Wittum TE. 2016. Extended spectrum β-lactam resistance in the enteric flora of patients at a tertiary care medical centre. *Zoonoses Public Health* 64:161–164.

López-Cerero L, Egea P, Serrano L, Navarro D, Mora A, Blanco J, Doi Y, Paterson DL, Rodríguez-Baño J, Pascual A. 2011. Characterisation of clinical and food animal *Escherichia coli* isolates producing CTX-M-15 extended-spectrum β-lactamase belonging to ST410 phylogroup A. *Int J Antimicrob Agents* 37:365–367.

Mohsin M, Raza S, Roschanski N, Schaufler K, Guenther S. 2016. First description of plasmid-mediated colistin-resistant extended-spectrum β-lactamase-producing *Escherichia coli* in a wild migratory bird from Asia. *Int J Antimicrob Agents* 48:463–464.

Mollenkopf DF, Cenera JK, Bryant EM, King CA, Kashoma I, Kumar A, Funk JA, Rajashekara G, Wittum TE. 2014. Organic or antibiotic-free labeling does not impact the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken. *Foodborne Pathog Dis* 11:920–929.

Mollenkopf DF, Weeman MF, Daniels JB, Abley MJ, Mathews JL, Gebreyes WA, Wittum TE. 2012. Variable within- and between-herd diversity of CTX-M cephalosporinase-bearing *Escherichia coli* isolates from dairy cattle. *Appl Environ Microbiol* 78:4552–4560.

Mora A, Herrera A, Mamani R, López C, Alonso MP, Blanco JE, Blanco M, Dahbi G, Garcia-Garrote F, Pita JM, et al. 2010. Recent emergence of clonal group O25b:K1:H4-B2-ST131 *ibeA* strains among *Escherichia coli* poultry isolates, including CTX-M-9-producing strains, and comparison with clinical human isolates. *Appl Environ Microbiol* 76:6991–6997.

NCBI (National Center for Biotechnology Information). 2016. *Basic local alignment search tool (BLAST)*. http://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed January 2016

Peirano G, Van Der Bij AK, Gregson DB, Pitout JDD. 2012. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. *J Clin Microbiol* 50: 294–299.

Razazi K, Derde LPG, Verachten M, Legrand P, Lesprit P, Brun-Buisson C. 2012. Clinical impact and risk factors for colonization with extended-spectrum β-lactamase-producing bacteria in the intensive care unit. *Intensive Care Med* 38:1769–1778.

Veldman K, Van Tulden P, Kant A, Testerink J, Mevius D. 2013. Characteristics of cefotaxime-resistant *Escherichia coli* from wild birds in The Netherlands. *Appl Environ Microbiol* 79:7556–7561.

Watson E, Jeckel S, Snow L, Stubbs R, Teale C, Wearing H, Horton R, Toszeghy M, Tearne O, Ellis-Iversen J, et al. 2012. Epidemiology of extended spectrum beta-lactamase *E. coli* (CTX-M-15) on a commercial dairy farm. *Vet Microbiol* 154:339–346.

Winokur PL, Brueggemann A, Desalvo DL, Hoffmann L, Apley MD, Uhlenhopp EK, Pfaller MA, Doern GV. 2000. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC β-lactamase. *Antimicrob Agents Chemother* 44:2777–2783.

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