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Wild Coastline Birds as Reservoirs of Broad-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* in Miami Beach, Florida

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A high rate of broad-spectrum-β-lactamase-producing *Escherichia coli* isolates was identified from seagull and pelican feces collected in the Miami Beach, Florida, area. The most commonly identified resistance determinants were CMY-2 and CTX-M-15. Those wild birds might be therefore considered vehicles for wide dissemination of multidrug-resistant *Enterobacteriaceae* in the United States.

Cephalosporin resistance in *Escherichia coli* is mostly mediated by production of extended-spectrum β-lactamases (ESBL) and plasmid-mediated AmpC-type cephalosporinases (15). During the past decade, CTX-M enzymes have been of growing importance worldwide, being reported widely in *Enterobacteriaceae* isolates recovered among humans (13, 14) either from community (*E. coli*) or nosocomial (*Klebsiella pneumoniae*) sources. In addition, CTX-M-positive *E. coli* has been identified in pets (4), in poultry (11), in cattle (20, 23), in retail meat (1, 20), and in wild animals (7), raising concerns regarding the transfer of ESBL between humans and animals. Among *Enterobacteriaceae*, CMY-2-type enzymes are the most commonly encountered plasmid-mediated AmpC-type β-lactamases worldwide and are involved in human hospital- or community-acquired infections (2, 24) but are also identified in isolates from cattle (8) or from retail meat (9). It has also been reported that seagulls might be a reservoir of multidrug-resistant bacteria (19, 21).

The objective of the study was to evaluate the occurrence of broad-spectrum-ß-lactam resistance determinants among *Enterobacteriaceae* recovered from wild bird feces collected at Miami Beach, Florida.

In April 2010, 53 fecal samples of wild seagulls (*Larus delawarensis*) and 10 fecal samples of pelicans were collected using a sterile spatula at different places on the shoreline of Miami Beach, Florida. Care was taken during sampling to avoid collection of beach sediment. Samples were placed in sterile tubes and processed in the laboratory. Samples were precultured in buffered peptone water (BPW) (Oxoid, Basingstoke, United Kingdom) at a dilution of 1/10 (wt/vol) and incubated at 37°C. Cultures were inoculated by streaking 10 μl of the suspensions onto ChromID ESBL agar (bioMérieux), which selects for broad-spectrum-cephalosporin-resistant isolates. The plates were incubated at 37°C overnight, and identification of *Enterobacteriaceae* isolates was performed by using an API20E system (bioMérieux, Marcy l'Etoile, France). Susceptibility testing was performed by disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France), as previously described (11). ESBL production was confirmed by a synergy test using disks containing cefotaxime and ticarcillin-clavulanate, and production of AmpC was evidenced by using Mueller-Hinton (MH) plates supplemented with cloxacillin (200 μg/ml) (11). The MICs were determined by Etest (AB bioMérieux, Solna, Sweden) on MH agar plates at 37°C (6). A total of 10 *Enterobacteriaceae* isolates displaying an ESBL phenotype were obtained from eight (14%) feces samples. All ESBL-producing isolates were resistant to ceftazidime and cefotaxime; all of them remained susceptible to carbapenems. Coresistances identified among the 10 ESBL-positive isolates were as follows: 70% were resistant to tetracycline, 80% to trimethoprim-sulfamethoxazole, 90% to nalidixic acid, 70% to ciprofloxacin, 60% to gentamicin, and 10% to chloramphenicol. Sixteen (29%) *E. coli* isolates displaying an AmpC-type phenotype were additionally identified. Among them, 44% were resistant to tetracycline, 25% to trimethoprim-sulfamethoxazole, 44% to nalidixic acid, 37% to ciprofloxacin, 19% to gentamicin, and 25% to chloramphenicol.

Detection of AmpC and ESBL genes was carried out by PCR (11). The purified PCR products were sequenced on both strands on an Applied Biosystems sequencer (ABI 377) and analyzed in the BLAST database (www.ncbi.nlm.nih.gov/blast/Blast.cgi). Eight of 10 (80%) ESBL producers were identified as *E. coli* and carried a $bla_{\text{CTX-M}}$-like gene, whereas two *Enterobacter cloacae* isolates ($n = 2$ [20%]) possessed a $bla_{\text{SHV-7}}$ gene. PCR and sequencing identified the CTX-M ESBL determinants as CTX-M-15 ($n = 5$ [50%]), CTX-M-32 ($n = 2$ [20%]), and a variant of CTX-M-2 ($n = 1$ [10%]), namely, CTX-M-124 (GenBank accession number JQ429324), which was identified as KluA-1 in *Kluyvera ascorbata*. All of the *E. coli* isolates displaying an AmpC-type phenotype produced the CMY-2 β-lactamase (Table 1). Only one AmpC-producing *K. pneumoniae* isolate, possessing the FOX-5-encoding gene, was identified. None of the isolates coproduced AmpC and ESBL enzymes.

Clonal diversity was assessed by pulsed-field gel electrophoresis (PGFE) as described previously (11). PGFE analysis showed a high diversity of genotypes (data not shown): 10 clones for 16 CMY-2-positive isolates, 4 clones for 5 CTX-M-15-positive iso-

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TABLE 1 Characteristics of the broad-spectrum β-lactamase-positive isolates[a]

Isolate	β-Lactamase	Genetic support of β-lactamase	Incompatibility group(s) of plasmid	Sequence type	Phylogenetic group	Clone
E. coli C22D	CTX-M-15	Plasmid	FIA + FIB	559	A	1
E. coli C26C	CTX-M-15	Plasmid	FIA + FIB	10	A	1
E. coli C37B	CTX-M-15	Chromosome	NT	405	D	2
E. coli C44B	CTX-M-15	Plasmid	FIA + FIB	410	A	3
E. coli C49E	CTX-M-15	Plasmid	FIA + FIB	617	A	4
E. coli C25E	CTX-M-32	Plasmid	FIB	1845	A	5
E. coli C33E	CTX-M-32	Plasmid	NT	853	A	6
E. coli P1 PALE	CTX-M-124	Chromosome	NT	648	D	7
E. cloacae C25D	SHV-7	Plasmid	L/M	ND	ND	8
E. cloacae C33G	SHV-7	Plasmid	L/M	ND	ND	8
E. coli C1	CMY-2	Plasmid	FIB	38	D	9
E. coli C43D	CMY-2	Plasmid	I1	38	D	10
E. coli C8A	CMY-2	Plasmid	I1	540	A	11
E. coli C9A	CMY-2	Plasmid	I1	167	A	12
E. coli C16A	CMY-2	Plasmid	I1	167	A	12
E. coli C19C	CMY-2	Plasmid	I1	162	B1	13
E. coli C23C	CMY-2	Plasmid	I1	963	D	14
E. coli C24A	CMY-2	Plasmid	I1	963	D	14
E. coli C29	CMY-2	Chromosome	NT	963	D	15
E. coli C30A	CMY-2	Plasmid	I1	963	D	14
E. coli C32A	CMY-2	Plasmid	I1	963	D	14
E. coli C35A	CMY-2	Plasmid	I1	963	D	14
E. coli P2 PINK	CMY-2	Plasmid	I1	224	B1	16
E. coli P1 PINK	CMY-2	Plasmid	NT	617	A	17
E. coli C46B	CMY-2	Plasmid	F	68	D	18
E. coli C47	CMY-2	Plasmid	F	68	D	18
K. pneumoniae C37C	FOX-5	Plasmid	A/C	ND	ND	ND

[a] ND, not determined; NT, not typeable using the PBRT method.

lates, 2 clones for 2 CTX-M-32-positive isolates, and 1 clone for 2 SHV-7-positive *E. cloacae* isolates (Table 1).

E. coli strains can be classified into four phylogenetic groups (A, B1, B2, and D). The virulent extraintestinal isolates belong mostly to group B2 and, to a lesser extent, to group D, whereas most commensal strains belong to groups A and B1 (5, 18). Phylogenetic grouping of *E. coli* isolates was determined by PCR as described previously (5). A total of 25% of the ESBL-positive *E. coli* strains and 63% of the CMY producers belonged to virulent extraintestinal *E. coli* group D. In contrast, 75% of the ESBL-producing and 25% of the CMY-producing *E. coli* strains belonged to phylogenetic group A, whereas 12% of the CMY-2 producers belonged to group B1, with both those latter groups corresponding to commensal strains (Table 1).

The MLST typing of the *E. coli* isolates was determined by sequencing seven essential genes (*adk*, *fumC*, *icd*, *purA*, *gyrB*, *recA*, and *mdh*) as described previously (22), followed by an analysis performed using the *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli/). MLST typing identified 16 different types among the 24 *E. coli* isolates (Table 1). The most commonly identified genotypes were ST963 ($n = 6$), ST38 ($n = 2$), ST617 ($n = 2$), ST167 ($n = 2$), and ST68 ($n = 2$), whereas isolates belonging to the ST559, ST1845, ST10, ST853, ST405, ST410, ST648, ST540, ST162, and ST224 genotypes were also identified. Different genotypes carrying the same ESBL determinant or CMY-2 were identified, and all the ST963 isolates produced CMY-2.

Analysis of plasmid content was performed for the isolates that tested positive for $bla_{\text{CTX-M}}$-like and bla_{CMY}-like genes as described previously (11). Incompatibility groups of ESBL- and CMY-positive plasmids were determined by PCR-based replicon typing as described previously (3). Interestingly, plasmid analysis performed on *E. coli* isolates always identified large plasmids for either the $bla_{\text{CTX-M}}$- or bla_{CMY}-positive isolates (data not shown). In addition, the majority of $bla_{\text{CMY-2}}$-positive plasmids belonged to the IncI1 incompatibility group ($n = 11$ [69%]), whereas the $bla_{\text{CTX-M}}$-positive plasmids mainly belonged to the IncF incompatibility group ($n = 5$ [50%]) (Table 1). The two $bla_{\text{SHV-7}}$-positive plasmids identified in two *E. cloacae* isolates belonged to the IncL/M group (Table 1).

Currently, infections with ESBL-producing bacilli occur not only in health care facilities but also in the community (16). Previous studies have reported multidrug resistance in wild birds (19, 21). The present report provides additional clues indicating that wild seagulls are carriers of ESBL-producing *E. coli*, as previously demonstrated in Europe (19, 21). We report here that the CTX-M-1 group (including ß-lactamases CTX-M-1, CTX-M-15, and CTX-M-32) was the main CTX-M group identified among birds residing on the coastline of Miami Beach, which mirrors previous findings corresponding to studies performed on the beaches of Porto, Portugal (21). This result fits also with the high prevalence of CTX-M-15 in community hospitals in the United States (16). Interestingly, this study reports a high proportion (29%) of CMY-positive *E. coli* strains among wild seagulls of Miami Beach, which correlated well with the high prevalence of the $bla_{\text{CMY-2}}$ gene among clinical or veterinary isolates (2, 8, 9, 24). It is noteworthy that the $bla_{\text{CMY-2}}$ gene was mostly located on the IncI1 plasmid, as reported previously (10, 12). The IncA/C plasmid, known to be widely associated with the $bla_{\text{CMY-2}}$ gene (12), was interestingly

not identified in this study. IS*Ecp1* was associated with most of the CTX-M- and CMY-positive isolates, highlighting the role of this insertion sequence in the dissemination of various β-lactamase genes, as previously described (2). Previous studies have reported the association of *E. coli* isolates of groups B2 and D with extraintestinal infections (18). No ESBL isolate belonged to those groups, whereas 63% of all CMY isolates belong to the D phylogroup. We did not identify *E. coli* strains with the ST131 type known to be frequently isolated in humans and frequently associated with CTX-M-15 (16). However, we identified here *E. coli* strains displaying ST10, ST38, ST405, ST617, and ST648, which were reported recently as the major sequence types among ESBL-producing *E. coli* strains involved in bacteremia in Canada (17). This report suggests that beaches may play a significant role for dissemination of various resistance determinants and may be a source of CTX-M-15- or CMY-related community-acquired infections. The role of wild birds traveling along the east coast of North America might therefore play a role in the dissemination of multidrug-resistant *E. coli* and *E. cloacae* strains.

Nucleotide sequence accession number. The sequence of CTX-M-124 has been deposited in GenBank under accession number JQ429324.

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