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Seagulls of the Berlengas Natural Reserve of Portugal as Carriers of Fecal *Escherichia coli* Harboring CTX-M and TEM Extended-Spectrum Beta-Lactamases▽

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***Escherichia coli* isolates containing the following extended-spectrum beta-lactamases have been detected in 11 of 57 fecal samples (19.3%) in Berlengas Island seagulls: TEM-52 (eight isolates), CTX-M-1 (one isolate), CTX-M-14a (one isolate), and CTX-M-32 (one isolate). Most of the extended-spectrum beta-lactamase-positive isolates harbored class 1 or class 2 integrons, which included different antibiotic resistance gene cassettes.**

The emergence and wide dissemination of extended-spectrum beta-lactamases (ESBLs) among clinical *Escherichia coli* isolates in hospitals in recent years are of great concern and represent a problem for the treatment of infectious diseases (19). It has also been reported that *E. coli* isolates containing ESBLs, mostly of the CTX-M class, are frequently detected in community patients (4) and have also been found in food-producing animals and household pets (2, 3, 5, 8, 11, 14, 17, 24). Moreover, a previous report identified ESBLs in fecal *E. coli* isolates of wild animals (9), mainly in birds of prey, but seagulls were not included in that study. ESBLs seem to be widely distributed in bacteria of different ecosystems, although more information is needed, especially for wild ecosystems. The purpose of our study was to analyze the carriage of ESBL-containing *E. coli* isolates in fecal samples of Berlengas Island seagulls and also to characterize the type of ESBLs and the phylogenetic groups of isolates. Berlengas Island is part of the Berlengas Natural Reserve, located 5.7 miles from the Portuguese coast, and it belongs to the National Network of Protected Areas. Fishermen inhabited the island in the past, but currently nobody lives there year round, although some tourists visit the island and a few people stay for vacations. Diverse species of seagulls make their nests on this island; in the last few years the population of seagulls has increased significantly and is considered a true plague (18).

Fifty-seven fresh seagull fecal droppings were obtained in different areas of the Berlengas Island during September 2007 and were tested for the presence of ESBL-containing *E. coli* isolates. Fecal samples were seeded in Levine agar plates supplemented with cefotaxime (CTX; 2 μg/ml), and colonies with typical *E. coli* morphology were selected and identified by classical biochemical methods and by the API 20E system (bioMérieux, La Balme Les Grottes, France). Susceptibility of the recovered *E. coli* isolates to 16 antibiotics (ampicillin, amoxicillin plus clavulanic acid, cefoxitin, CTX, ceftazidime, aztreonam, imipenem, gentamicin, amikacin, tobramycin, streptomycin, nalidixic acid, ciprofloxacin, sulfamethoxazole-trimethoprim, tetracycline, and chloramphenicol) was tested by the disk diffusion method (7). *E. coli* ATCC 25922 was used as a quality control strain. Broad-spectrum cephalosporin-resistant isolates were selected for further studies (one isolate per sample), and they were screened for ESBL production according to the CLSI criteria (7).

The presence of genes encoding TEM, SHV, OXA, CTX-M, and CMY type beta-lactamases was studied by specific PCRs (1, 13, 23). All obtained amplicons were sequenced on both strands, and sequences were compared with those included in the GenBank database and in the website http://www.lahey.org/Studies/ to identify the beta-lactamase genes. The genetic environment of $bla_{\text{CTX-M}}$ genes was also tested by PCR and by sequencing with previously reported primers (10, 15, 22).

The presence of other antibiotic resistance genes, associated with chloramphenicol (*cmlA*), tetracycline (*tetA* and *tetB*), streptomycin (*aadA*), and sulfonamide (*sul1*, *sul2*, and *sul3*) resistance, among our isolates was also analyzed by PCR and sequencing (21). The presence of the *intI1* and *intI2* genes, encoding class 1 and 2 integrases, respectively, and the composition of the variable regions of class 1 and 2 integrons were studied by PCR and sequencing (21). The identification of the major phylogenetic groups among our isolates was determined by PCR (6). Positive and negative controls from the bacterial collection of the University of La Rioja, Logroño, Spain, were used in all assays.

E. coli isolates were detected in Levine CTX plates from 11 of the 57 (19.3%) fecal samples studied. All 11 isolates obtained from these samples were intermediate or resistant to CTX and/or ceftazidime and had a positive screening test for ESBL production. Only 1 of the 11 *E. coli* isolates (GV-

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TABLE 1. Characteristics of the ESBL-positive fecal *E. coli* isolates recovered from seagulls of Berlengas Island in Portugal

E. coli isolate	Phylogenetic group	Non-beta-lactams to which isolates were resistant[a]	Type of ESBL	Class 1 integrons		Class 2 integrons		Other gene(s) detected
				Presence of *intI1*	Gene cassettes	Presence of *intI2*	Gene cassettes	
GV-5	D	NAL, CIP, TET, STR, SXT, CHL	TEM-52	+	*dfrA1* + *aadA1*	−		*tetA*, *sul1*, *sul3*, *cmlA*
GV-6	B1	NAL, CIP, TET, STR	TEM-52	−		+	*dfrA1* + *sat* + *aadA1*	*tetA*
GV-8	B2	NAL, CIP, TET, STR, SXT, CHL	TEM-52	+	*sat* + *psp* + *aadA*[b]	+	*dfrA1* + *sat* + *aadA1*	*tetA*, *sul2*, *sul3*, *cmlA*
GV-9	B1	NAL, CIP, TET, STR, SXT, CHL	TEM-52	+	*dfrA1* + *aadA1*	−		*tetA*, *sul1*, *sul2*, *sul3*, *cmlA*
GV-33	A	NAL, TET	TEM-52	−		−		*tetB*
GV-51	B1	NAL, CIP, TET, SXT	TEM-52	−		+	*dfrA1* + *sat* + *aadA1*	*tetA*, *sul2*
GV-52	A	NAL, CIP, TET	TEM-52	−		+	*dfrA1* + *sat* + *aadA1*	*tetA*
GV-54	D	NAL, CIP, TET, STR, SXT	TEM-52	−		+	*dfrA1* + *sat* + *aadA1*	*tetB*, *sul2*
GV-23	A	TET, CHL	CTX-M-1	+	bla_{OXA-1} + *aadA1*	−		*tetB*, *su1l*
GV-10	A	NAL, TET, STR	CTX-M-14a	−		−		*tetB*, *aadA*
GV-12	B1	NAL, CIP, TET, STR	CTX-M-32	+	*sat* + *aadA1*[b]	−		*tetB*

[a] TET, tetracycline; CHL, chloramphenicol; NAL, nalidixic acid; CIP, ciprofloxacin; STR, streptomycin; SXT, trimethoprim-sulfamethoxazole.
[b] The *qacE*Δ-plus-*sul1* 3′ conserved region was absent in two *intI1*-positive *E. coli* isolates containing bla_{TEM-52} and $bla_{CTX-M-32}$.

10) showed resistance to amoxicillin plus clavulanic acid. The beta-lactamase genes detected in these isolates were the following (numbers of isolates are in parentheses): bla_{TEM-52} (8), $bla_{CTX-M-1}$ plus bla_{OXA-1} (1), $bla_{CTX-M-14a}$ (1), and $bla_{CTX-M-32}$ (1) (Table 1). It is interesting that 73% of the ESBL-positive isolates of seagulls harbored the bla_{TEM-52} gene and that 27% of the isolates harbored the bla_{CTX-M} gene. A high prevalence of TEM-52 has also been recently observed in *E. coli* isolates from healthy food-producing animals and chicken meat products from Portugal (16).

Different genetic environments surrounding the bla_{CTX-M} genes were detected (Fig. 1). The sequence of the fragment obtained by PCR upstream of the $bla_{CTX-M-1}$ gene in the *E. coli* GV-23 strain revealed the presence of a region of the IS*26* transposase flanking a partially truncated IS*Ecp1* followed by an intergenic region; this whole structure has been previously found in *E. coli* (13, 22). The presence of IS*Ecp1* and IS*903* surrounding the $bla_{CTX-M-14a}$ gene in *E. coli* GV-10 was identified, and the genetic environment of the $bla_{CTX-M-32}$ gene detected in *E. coli* GV-12 included IS*Ecp1*/IS*5* upstream of the *bla* gene and *orf477* downstream, as also detected by others (25).

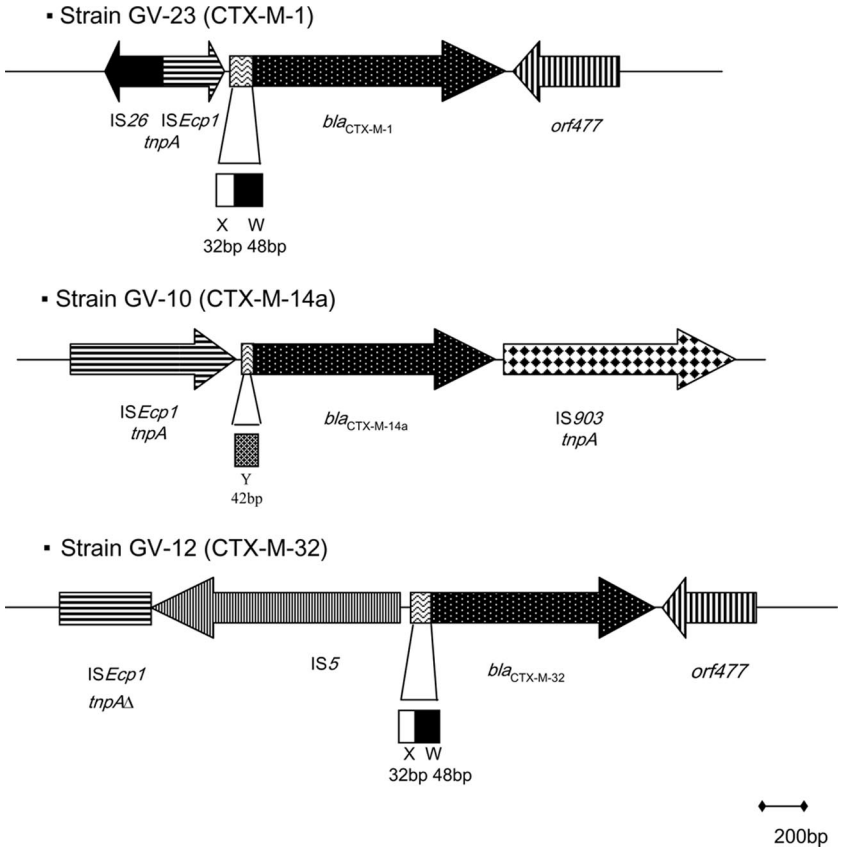

FIG. 1. Genetic environment of bla_{CTX-M} genes in three *E. coli* isolates recovered from seagull fecal samples (the intergenic X, Y, and W regions have been previously reported [10]).

A variety of resistance genes (*cmlA*, *tetA*, *tetB*, *aadA*, *sul1*, *sul2*, and *sul3*) were observed among our ESBL-producing *E. coli* isolates (Table 1). Five isolates harbored class 1 integrons with the following gene cassettes in their variable regions: *dfrA1* plus *aadA1* (two isolates), *sat* plus *psp* plus *aadA2* (one isolate), *sat* plus *aadA1* (1 isolate), and $bla_{\text{OXA-1}}$ plus *aadA1* (one isolate). Five isolates harbored class 2 integrons, and the gene cassette arrangement *dfrA1* plus *sat* plus *aadA1* was identified in all of them. *E. coli* GV-8 contained simultaneously class 1 and 2 integrons. Eight of the ESBL-positive isolates corresponded to the A and B1 phylogenetic groups, two isolates corresponded to the D group, and only one $bla_{\text{TEM-52}}$ isolate was assigned to the B2 phylogenetic group (Table 1). Previous studies have reported the association of *E. coli* isolates of the B2 group with extraintestinal infections (20), and the fecal origin of our isolates could explain the low prevalence of this phylogroup.

It is important to note the high prevalence and moderate diversity of ESBLs detected in fecal *E. coli* from seagulls that inhabit a natural reserve, as is the case for Berlengas Island. As previously indicated, the population of seagulls on an island that is not too far away from the Portuguese coast has significantly increased in recent years. The possibility that these animals eat the remains of human food cannot be excluded. This study gives new evidence for the wide dissemination of ESBLs in *E. coli* isolates from wild animals, as is the case for seagulls. More studies of this nature should be performed in the future to analyze the prevalence of this type of resistant bacteria in different ecosystems.

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