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EPIDEMIOLOGY AND SURVEILLANCE

Prevalence of Extended-Spectrum-β-Lactamase- and/or Carbapenemase-Producing *Escherichia coli* Isolated from Yellow-Legged Gulls from Barcelona, Spain

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ABSTRACT Seventy-two (54.5%) out of 132 fecal samples from a group of yellow-legged gulls in Barcelona, Spain, were positive for *Escherichia coli* producing either extended spectrum beta-lactamases (ESBL) (51.5%), carbapenemase (1.5%), or cephamycinase (1.5%). The isolation of two carbapenemase-producing *E. coli* strains is a matter of concern.

KEYWORDS carbapenemases, *E. coli*, ESBL

In the last decade, the number of bacterial pathogens presenting multidrug resistance to antibacterial agents has increased dramatically, becoming an emergent global AQ: A concern and a major public health problem (1). The main cause behind the increasing rates of resistance can ultimately be found in the abuse and misuse of antibacterial agents, whether used in patients and livestock or released into the environment. Once antimicrobial-resistant bacteria emerge, they can spread locally or globally. The main factors contributing to their spread at a global level comprise migrant birds, globalization of commercial food, and international travel.

There have been several studies about the presence of resistant bacteria in gulls (2, 3), to the extent of being considered an indicator of environmental antibiotic resistance occurrence, as they are distributed almost all around the world (4). Meerburg et al. (5) showed that gulls feces contain a greater average concentration of *E. coli* than other wild animals, and according to Stedt et al. (4), Spain is the country in Europe with the highest levels of gull *E. coli* isolates resistant to ≥1 antibiotic.

The objective of this study was to investigate the prevalence of extended-spectrum beta-lactamase (ESBL)- and/or carbapenemase-producing *Enterobacteriaceae* from fecal swabs obtained from a group of yellow-legged gulls (*Larus michahellis*) in Barcelona, Spain.

The study was conducted from the beginning of May to late July 2014 in the city of Barcelona, including the breeding period of the yellow-legged gull in the city. The sampling program was part of the sanitary and epidemiological surveillance that is carried out by the Public Health Agency, Barcelona, the institution responsible for the supervision and surveillance of the species. The sampling sites were chosen according to citizens' reports regarding the species nesting on their terraces or high roofs of the F1 city. Every gull chick from each nest found (Fig. 1) was sampled, which amounts to 132 samples in total. All samples were obtained from young specimens born in that same year, and all nests were independent from each other, since the urban structure of cities

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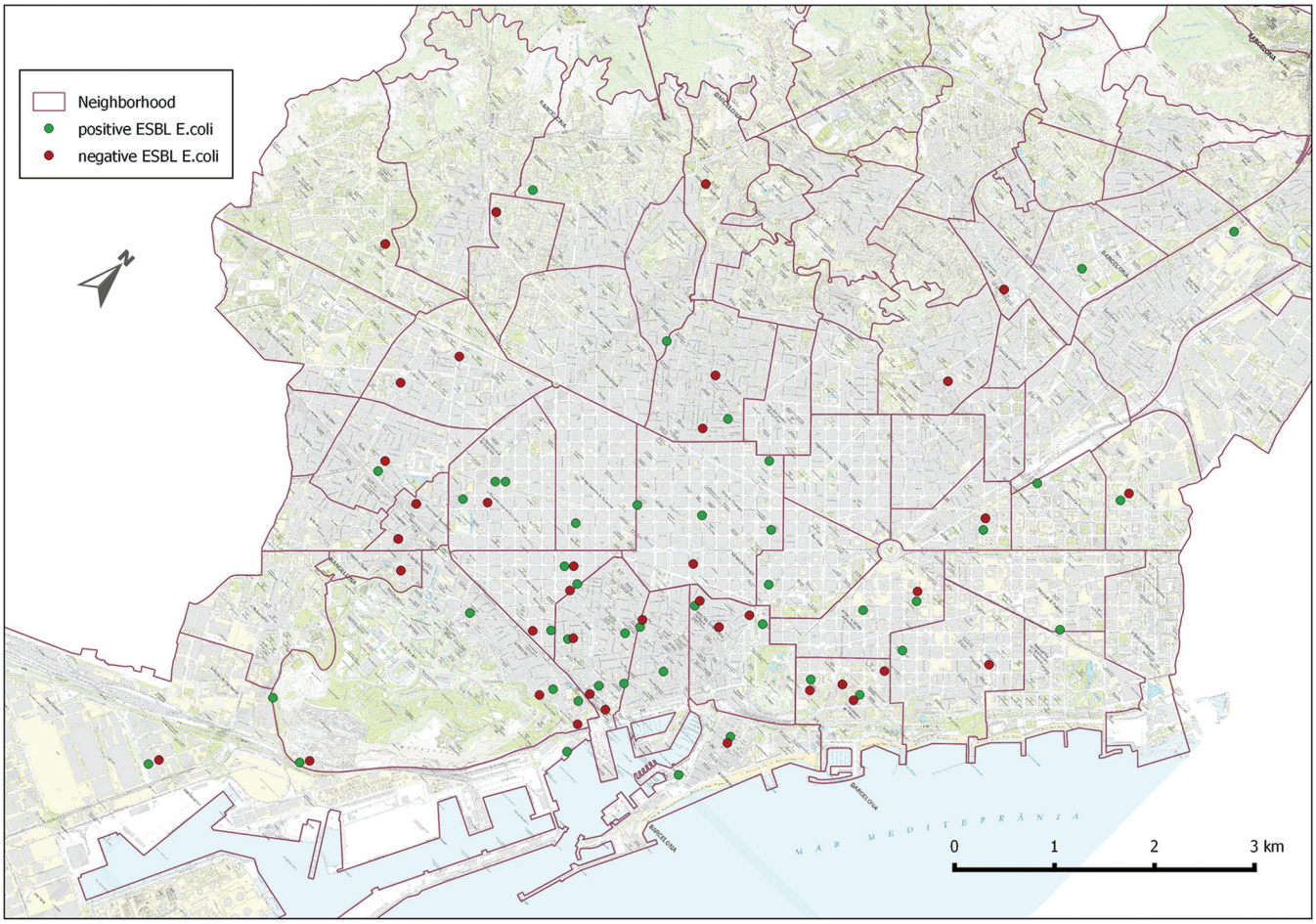

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FIG 1 Location of sites where positive (green) and negative (red) samples were collected (Barcelona, Spain).

promotes isolated instead of colonial nesting. Fecal material was obtained by sampling the cloacae of gull chicks with sterile swabs. Each swab was individually preserved in Cary-Blair medium at 2 to 8°C and analyzed within 24 h in the laboratory of the Public Health Agency, Barcelona.

The samples were plated on ESBL chromogenic agar (bioMérieux, France), and burgundy red colonies were selected, according to the manufacturer's instructions. Colonies were further identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Inc., Bremen, Germany). Susceptibility to ampicillin, amoxicillin-clavulanic acid, cefuroxime, ceftriaxone, cefotaxime, meropenem, gentamicin, amikacin, nalidixic acid, and ciprofloxacin was determined by the disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoints (version 5.0, 2015 6). The double-disk diffusion technique was performed for phenotypic ESBL detection. The presence of carbapenemases was assessed with the modified Hodge test, according to phenotypic susceptibility results. Characterization of ESBL and carbapenemases genes was performed by PCR, followed by DNA sequencing (7) (for ESBL, the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes; for carbapenemases, the bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{NDM} genes; and for cephamycinases, the bla_{CMY}, bla_{DHA}, bla_{FOX}, bla_{ACC}, bla_{EBC}, and bla_{MOX} genes). Seventy-two (54.5%) out of 132 fecal samples were positive for *Escherichia coli* producing either ESBL (68/132 [51.5%]), carbapenemase (2/132 [1.5%]), or cephamycinase (2/132 [1.5%]) (Table 1), with SHV being the most prevalent group (38/132 [28.8%]). Forty-five strains (62.5%) were resistant to quinolones, 22 strains (30.6%) were resistant to gentamicin, and 9 strains (12.5%) were resistant to amikacin.

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TABLE 1 Distribution of β-lactamases

β-Lactamase(s)	No. of strains	%
SHV group	38	52.8
SHV-12	24	33.3
SHV-12 + TEM-1	13	18
SHV-2	1	1.4
CTX-M group	30	41.6
CTX-M-15	11	15.3
CTX-M-15 + TEM-1	2	2.8
CTX-M-1	1	1.4
CTX-M-1 + TEM-1	5	6.9
CTX-M-1 + TEM-84	1	1.4
CTX-M-14	4	5.5
CTX-M-14 + TEM-1	6	8.3
VIM-1 + KPC-2	2	2.8
CMY-2	2	2.8
Total	72	100

Repetitive extragenic palindromic-PCR (rep-PCR) showed a high genetic heterogeneity among the strains with up to 57 different clones, with 15 of them containing two different isolates each (data not shown). Agglutination with antiserum O:25 was used to identify CTX-M-15-producing isolates belonging to the high-risk clone O:25b-ST131, but all isolates were negative.

Of note, two *E. coli* isolates carried both the bla_{KPC-2} and bla_{VIM} genes for carbapenem resistance. Multilocus sequence typing (MLST) (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) and PCR-based phylogroup analysis (8) identified them as belonging to the sequence type 1011 (ST1011) phylogroup E (strain 40) and ST354 phylogroup F (strain 71), respectively, which have been previously reported in human strains (9, 10). The genetic transference of carbapenemase genes was tested by biparental mating experiments using *E. coli* J53 AziR as the recipient strain. Transconjugants were selected in Mueller-Hinton agar plates containing 100 μg/ml sodium azide and 1 μg/ml meropenem (Sigma Chemical Co., St. Louis, MO). Successful conjugation was confirmed by specific PCR amplification. Table 2 shows the MICs determined by Etest of different

TABLE 2 *In vitro* susceptibilities of original strains of *E. coli* and *E. coli* transconjugants expressing VIM-1 and/or KPC-2 (Etest, EUCAST)

	MIC (μg/ml)						
	Original strains			Transconjugants			
Antibiotic	40 (VIM-1/KPC-2)[a]	71 (VIM-1/KPC-2)[b]	J53[c]	J53 40T3 (KPC-2)[d]	J53 40T5 (VIM-1/KPC-2)[e]	J53 71T1 (VIM-1)[f]	J53 71T3 (VIM-1/KPC-2)[g]
Cefoxitin	>256	>256	2	8	256	256	64
Cefotaxime	32	24	0.094	0.75	16	16	64
Ceftazidime	256	96	0.125	1	64	64	96
Imipenem	4	24	0.19	1.5	3	1	12
Meropenem	4	32	0.023	0.75	0.5	0.25	8
Ertapenem	6	12	0.008	0.38	0.38	0.64	4
Aztreonam	16	128	0.064	12	16	0.25	256
Ciprofloxacin	>32	>32	0.064	0.047	0.064	0.064	0.064
Gentamicin	>32	>32	0.25	0.25	1	2	2
Amikacin	3	3	1	1	1.5	1.5	1.5
Tobramycin	16	12	0.125	0.125	3	3	3
Colistin	0.25	0.125	0.125	0.25	0.125	0.19	0.25

[a] *E. coli* strain 40 isolated from a yellow-legged gull.
[b] *E. coli* strain 71 isolated from a yellow-legged gull.
[c] Sodium azide-resistant *E. coli* J53 strain used as a recipient in the conjugation experiment.
[d] *E. coli* transconjugant obtained from strains 40 and J53 that received only bla_{KPC-2}.
[e] *E. coli* transconjugant obtained from strains 40 and J53 that received both bla_{KPC-2} and bla_{VIM-1}.
[f] *E. coli* transconjugant obtained from strains 71 and J53 that received only bla_{VIM-1}.
[g] *E. coli* transconjugant obtained from strains 71 and J53 that received both bla_{KPC-2} and bla_{VIM-1}.

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antibiotics for the original and transconjugant strains with the corresponding carbapenemases harbored.

Plasmid analysis by S1 nuclease–pulsed-field gel electrophoresis (PFGE) (7) and replicon typing (7) were then performed on both the original strains and transconjugants to determine the size of these plasmids and classify them within the incompatibility groups. Digoxigenin-labeled probes for the bla_{VIM} and bla_{KPC} genes were hybridized against blotted nylon membranes from the S1-PFGE gels. The bla_{KPC-2} gene was located in a plasmid ca. 60 kb in size in strain 40 and in a plasmid of <50 kb in strain 71, both being nontypeable plasmids. The genetic environment of the bla_{KPC-2} genes was determined by inverse PCR, leading to the identification of an IS*Kpn27*-ΔTEM-bla_{KPC-2}-IS*Kpn6-kor*C genetic arrangement, which was similar to those previously described among different isolates of human origin from China and Taiwan (11, 12). Further analysis based on next-generation sequencing is needed to describe additional elements of these plasmids for a more robust analysis.

The bla_{VIM-1} gene was located in both strains in an In*3103* class I integron, carried in a ca. 100-kb plasmid belonging to the incompatibility group I1-Iγ. This integron also contained an aminoglycoside 6′-*N*-acetyltransferase (*aacA4*) gene and a 3′-(10)-*O*-adenylyltransferase (*aadA1*) gene. The presence of the bla_{VIM-1} gene within an In3103 class I integron was also described by at least one report in Spain, albeit in that case, it was located in a nontypeable plasmid of ca. 60 kb and recovered from a human patient (13).

Our data showed a higher percentage of resistant *E. coli* in gull fecal samples than in previous studies (14–16), but it also represents the first study, to our knowledge, reporting the coexistence of two carbapenemase genes in *E. coli* recovered from yellow-legged gulls. However, some OXA-48-producing *E. coli* strains could have been lost due to the methodology that was specifically designed to search for ESBL. The fact that carbapenem-resistant isolates recovered from the fecal samples of gulls share the same sequence types and resistance modules as those recovered from human samples in different parts of the world highlights the potential role of migratory birds in the dissemination and spread of antibiotic resistance genes.

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