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gyrA Mutations Associated with Nalidixic Acid-Resistant Salmonellae from Wild Birds

The emergence of quinolone-resistant isolates of salmonellae has been related to the use of antibiotics in veterinary medicine, which causes an important impact on the selection of resistance (2, 3, 8). It has also been demonstrated that veterinary clones possess the same mechanism of resistance as human clinical isolates (10). Quinolone resistance in salmonellae is mainly associated with mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* and *parC* genes (6, 10).

However, little is known about what the incidence of resistance in wild animals is and which of the mechanisms of resistance these strains have, although it is thought that wild animals are also integrated in the same epidemiologic cycle (1). The aim of this study was to analyze the prevalence of quinolone-resistant isolates of salmonellae from wild birds and determine the mechanisms involved.

A total of 45 *Salmonella enterica* subsp. *enterica* isolates were obtained from stool samples from wild birds just arrived to GREFA Wild Life Hospital. Seven strains (15.5%) were selected on the basis of resistance to nalidixic acid. The MICs of nalidixic acid (Sigma Aldrich, Madrid, Spain), ciprofloxacin, and enrofloxacin (Bayer, Leverkusen, Germany) were measured by a twofold agar dilution method and interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards guidelines (4). To identify *gyrA* and *parC* mutations in resistant isolates, PCR and direct DNA sequencing were performed as follows. A 312-bp fragment of the QRDR of the *gyrA* gene was amplified from a genomic DNA template by using specific primers gyr A-1 (5′-GGTACACCGTGCCGTACTTT-3′) and gyr A-2 (5′-TCCA CGAAATCCACCGTC-3′) corresponding to positions 17 to 137 and 311 to 328, respectively. These salmonella-specific primers were constructed on the basis of *Salmonella enterica* serotype Typhimurium *gyrA* gene sequence data (GenBank accession number X78977). The primers for the amplification of the QRDR of the *parC* gene have been described elsewhere (9). The mutations in the *gyrA* gene leading to the amino acid substitutions and MICs are shown in Table 1.

Strains S2, S9, S10, S14, and S20 carried a Cys-284→Ala transversion, resulting in a Ser-83→Tyr substitution in the GyrA subunit. This mutation has been described in *Salmonella* serotype Typhimurium strains resistant to nalidixic acid and reduced the susceptibility to fluoroquinolones isolated from food-producing animals (10). Strains S57 and S60 carried a Gly-259→Cys transition, resulting in an Asp-87→Asn substitution in the GyrA subunit. This mutation has also been described in *Salmonella* isolates of human and animal origin (2, 5).

No mutations in the QRDR of *parC* were detected. These results may confirm that *parC* mutations are not necessary to obtain a high level of resistance to nalidixic acid (7).

The mechanisms of resistance described here are the same as the ones described for humans and domestic animals. Therefore, it is possible that wild animals be included in the epidemiologic cycle of the spread of resistance and the dissemination of resistant bacteria among animals and the environment. Further studies are necessary to obtain more information about what is the real role of wild animals in these facts.

Nucleotide sequence accession numbers. The GenBank accession numbers for the partial sequences of the quinolone-resistant strains are AF447053, AF447054, AF447055, AF447056, AF447057, AF447058, and AF447059.

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TABLE 1. Susceptibility to quinolone and GyrA substitutions of *S. enterica* subsp. *enterica* isolates from wild birds

Isolate	Serovar	Origin	MIC (μg/ml)[a]			Position	Mutation (amino acid)
			NAL	CIP	ENR		
S2	Havana	Black vulture	256	0.25	1	83	TCC (Ser)\TAC (Tyr)
S9	Adelaide	Sparrow hawk	256	0.25	1	83	TCC (Ser)\TAC (Tyr)
S14	Adelaide	Imperial eagle	256	0.25	1	83	TCC (Ser)\TAC (Tyr)
S20	Adelaide	Little owl	256	0.25	1	83	TCC (Ser)\TAC (Tyr)
S10	Brandenburg	Kestrel	512	0.25	1	83	TCC (Ser)\TAC (Tyr)
S57	Hadar	Kestrel	512	0.25	1	87	GAC (Asp)\CAC (Asn)
S60	Hadar	Jay	512	0.25	1	87	GAC (Asp)\CAC (Asn)

[a] NAL, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin.

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