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Molecular mechanisms of quinolone resistance in clinical isolates of *Aeromonas caviae* and *Aeromonas veronii* bv. *sobria*

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Summary. Mutations in quinolone targets were studied together with quinolone efflux pump activation and plasmid-mediated quinolone resistance determinants in nalidixic-acid-resistant isolates of *Aeromonas caviae* and *Aeromonas veronii*. Among 135 clinical *Aeromonas* spp. isolated from stools of patients with gastrointestinal symptoms, 40 nalidixic acid-resistant strains belonging to *A. caviae* and *A. veronii* were selected and their susceptibility to different quinolones (ciprofloxacin, norfloxacin, ofloxacin) further evaluated. Susceptibility to nalidixic acid and ciprofloxacin in the presence/absence of Phe-Arg-β-naphthylamide was also determined. The 16 nalidixic-acid-resistant strains identified as *A. caviae* were more resistant than the 24 *A. veronii* bv. *sobria* strains to ciprofloxacin, norfloxacin, and ofloxacin. All strains showed a mutation (single or double) at position 83 of the QRDR sequence of *gyrA*, with Ser-83 → Ile as the most frequent substitution. By contrast, no mutations were found at position 87 of *gyrA*. Double substitutions (GyrA-ParC) were detected in 50% of *A. veronii* bv. *sobria* isolates and in 43.75% of *A. caviae* strains. Both species showed decreases in the MICs of ciprofloxacin. A *qnr*S gene was found in an *A. caviae* strain. Thus, in the two species of nalidixic-acid-resistant *Aeromonas* isolates examined, resistance mediated by efflux pumps contributed only slightly to ciprofloxacin resistance. While two isolates were positive for the *aac(6´)-Ib* gene, no *-cr* variants were detected. [**Int Microbiol** 2010; 13(3):135-141]

Keywords: *Aeromonas* spp. · quinolone resistance · nalidixic acid resistance · ciprofloxacin

Introduction

Mesophilic motile *Aeromonas* is a normal inhabitant of freshwater. In humans, these bacteria cause opportunistic and extraintestinal infections as well as gastroenteritis. Of the 16 *Aeromonas* phenospecies now recognized, *A. hydrophila*, *A. caviae*, *A. veronii*, and *A. trota* are those most frequently implicated in human infectious diseases [11].

Quinolones are broad-spectrum antibacterial agents but their extensive use in human and veterinary medicine has resulted in rising levels of quinolone resistance. The two main mechanisms of quinolone resistance are chromosomally encoded and consist of: (i) modification of quinolone targets with changes in the DNA gyrase (*gyrA*) and/or topoisomerase IV (*parC*) genes, and (ii) a decreased intracellular concentration due to impermeability of the membrane or to overexpression of efflux pump systems [20]. Recently, however, plasmid-mediated quinolone resistance has been described in *Enterobacteriaceae* and in other bacterial families, such as *Aeromonadaceae* and *Vibrionaceae* [12,13b].

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The plasmid-borne *qnr* genes, which currently comprise five families, *qnrA*, [4] *qnrB* [4], *qnrC* [27], *qnrD* [6], and *qnrS* [4], encode pentapeptide repeat proteins that block the action of ciprofloxacin on bacterial DNA gyrase and topoisomerase IV. This results in low-level quinolone resistance and provides a favorable background for higher resistance at quinolone concentrations that would be lethal in their absence, through secondary changes in DNA gyrase and topoisomerase IV, porin or efflux systems [10]. Other plasmid-mediated quinolone resistance determinants have been reported as well: aminoglycoside acetyltranferase AAC(6´)-Ib-cr [19], QepA [15], and the recently described plasmid-encoded efflux pump OpxAB [12].

Quinolone-resistant enteric pathogens have emerged and their spread has been facilitated through the uncontrolled use of the quinolone group of antibiotics [23]. The prevalence and molecular mechanisms of resistance of *A. caviae* and *A. veroni* bv. *sobria* have been described [24,26]. The aim of this study was to evaluate the susceptibility to different quinolones of the two *Aeromonas* species isolated in our laboratory from stools of patients with diarrhea, focusing on the molecular and plasmid-mediated mechanisms of quinolone resistance. Although a plasmid-mediated quinolone resistance determinant was described in a previous report, our study provides a more detailed description of the prevalence of quinolone resistance, especially in clinical isolates of the *Aeromonas* species.

In order to determine whether molecular- and plasmid-mediated mechanisms are involved in *Aeromonas* spp. quinolone resistance, the presence and nature of chromosomally encoded mutations and the emergence of plasmid-mediated quinolone resistance was evaluated among clinical *Aeromonas* spp. identified as *A. veronii* and *A. caviae* by molecular techniques. The isolates came from stools of patients with gastrointestinal symptoms [1].

Materials and methods

Between 2000 and 2007, 40 nalidixic-acid-resistant isolates of *Aeromonas* spp. were selected among 369 isolates of *Aeromonas* spp. collected from stools samples obtained from patients with diarrhea. Identification to the genus level and susceptibility to antibiotics were determined using the semi-automatic WIDER I system (Soria Melguizo, Madrid, Spain). Minimum inhibitory concentrations (MICs) of fluoroquinolones, including ciprofloxacin, norfloxacin, and ofloxacin (Sigma-Aldrich, St. Louis, MO, USA), were determined by broth microdilution according to the Clinical Laboratory Standards Institute [7]. *Escherichia coli* ATCC 25922 was used as the control

Table 1. Primers used in this study

Primer	Sequence (5´-3´)	Gene	Size of PCR-amplified product (bp)	Reference
QnrSm-F	5´-GCAAGTTCATTGAACAGGGT-3´	*qnrS1* to *qnrS2*	428	[5]
QnrSm-R	5´-TCTAAACCGTCGAGTTCGGCG-3´	*qnrS1* to *qnrS2*		
QnrA-F	5´-AGAGGATTTCTCACGCCAGG-3´	*qnrA*	580	[5]
QnrA-R	5´-TGCCAGGCACAGATCTTGAC-3´	*qnrA*		
QnrB-F	5´-GGAATCGAAATTCGCCACTG-3´	*qnrB*	264	[5]
QnrB-R	5´-TTTGCCGTCCGCCAGTCGAA-3´	*qnrB*		
GyrA-F	5´-TCCTATCTTGATTACGCCATG-3´	*gyrA*	441	[9]
GyrA-R	5´-CATGCCATACCTACCGCGAT-3´	*gyrA*		
ParC-F	5´-CGGAATGCCAGGAGAAAGA-3´	*parC*	204	[9]
ParC-R	5´-GGTCATGATGATGATGTTTG-3´	*parC*		
QepA-F	5´-GCAGGTCCAGCAGCGGGTAG-3´	*qepA*	218	[13]
QepA-R	5´-CTTCCTGCCCGAGTATCGTG-3´	*qepA*		
Aac6´-F	5´-TTGCGATGCTCTATGAGTGGCTA-3´	*aac6´*	482	[14]
Aac6´-R	5´-CTCGAATGCCTGGCGTGTTT-3´	*aac6´*		
16S rRNA-F	5´-AGAGTTTGATCATGGCTCAG-3´	16S rRNA	1502	[2]
16S rRNA-R	5´-GGTTACCTTGTTACGACTT-3´	16S rRNA		
GyrB-F	5´- TCCGGCGGTCTGCACGGCGT-3´	*gyrB*	1100	[28]
GyrB-R	5´-TTGTCCGGGTTGTACTCGTC-3´	*gyrB*		

Table 2. Resistance phenotypes, MICs to nalidixic and ciprofloxacin, and number of strains belonging to *Aeromonas caviae* and *A. veronii* species carrying substitutions in *gyrA* and *parC*

					Ciprofloxacin MICs (mg/l)				Nalidixic acid MICs (mg/l)			
Phenotype	Species	GyrA	No. of strains	ParC	50%	90%	Range	Mean	50%	90%	Range	Mean
NalR; CipR	*A. veronii*	Val-83	1	NF	–	–	4	–	–	–	512	–
	A. caviae	Ile-83	2	Ile-80(1); (*)Thr-80 (1)	–	–	4 – >64	–	–	–	128–512	–
		Arg-83	1	NF	–	–	64	–	–	–	1024	–
NalR; CipI	*A. veronii*	Ile-83	8	Ile-80(4); Arg-80(2)	–	–	2	–	256	256	16–512	240
		Val-83	1	NF	–	–	2	–	–	–	256	–
	A. caviae	Ile-83	3	Ile-80(3)	–	–	2	–	–	–	128–256	–
NalR; CipS	*A. veronii*	Ile-83	12	Ile-80(4); Arg-80(2)	0.5	1	>0.12–1	–	128	256	32–256	167.83
		Val-83	1	NF	–	–	1	–	–	–	128	–
		Arg-83	1	NF	–	–	1	–	–	–	256	–
	A. caviae	Ile-83	8	Ile-80(1); Arg-80(1)	0.5	1	0.25–1	0.8	256	512	32–512	324
		Val-83	1		–	–	0.25	–	–	–	128	–
		Arg-83	1		–	–	1	–	–	–	128	–

Nal, nalidixic; Cip, ciprofloxacin; Val, valine; Ile, isoleucine; Arg, arginine; Thr, threonine; NF, not found.
(–) The $MIC_{50,}$ $MIC_{90,}$ and geometric mean were not calculated when there were <8 isolates. (*) Isolate harboring the *qnrS2* gene.

strain. Dilutions of ciprofloxacin, norfloxacin, and ofloxacin ranged from 0.12 to 64 mg/l, and the results were interpreted according to CLSI M100-S18 guidelines [7]. Susceptibility to nalidixic acid and ciprofloxacin in the presence and absence of 20 mg of the efflux pump inhibitor Phe-Arg-β-naphthylamide/l was examined using the E-test (AB Biodisk, Solna, Sweden).

Final identification of the strains was carried out using conventional biochemical techniques and by amplification of the 16S rRNA gene (a 1502-bp PCR amplicon) with a set of previously described primers [2], followed by *Eco*105I and *Mbo*I digestion of the PCR amplicons [8]. The 16S ribosomal DNA amplicons were also sequenced, and the sequences compared with those in the GenBank database using the BLAST program available at the National Center for Biotechnology Information [www.ncbi.nlm.nih.gov]. The accession numbers of the wild-type strains used for comparison purposes were DQ298051.1 for *A. caviae*, EF631963.1 for *A. veronii* bv. *sobria*, and FJ233864.1 for *A. veronii* bv. *veronii*. The *gyrB* gene, a molecular marker for phylogenetic inference in the genus *Aeromonas*, was analyzed to confirm 16S ribosomal DNA identification in strains whose 16S RNA gene RFLP-patterns and 16S RNA sequences did not coincide with the results of the biochemical test [28]. Accession numbers for the wild-type *gyrB* sequences used for comparison were EF 064800 for *A. caviae*, and AY10179 and FJ238508 for *A. veronii*.

Mutational alterations in QRDR were studied by PCR using primers previously described [9]. The QRDR sequences obtained for *gyrA* and *parC* genes were compared with the sequences of the *A. caviae* complex (GenBank accession numbers were AY027899 and AF435418, respectively), and the *A. sobria* complex (GenBank accession numbers AY027900 and AF435420, respectively). The *qnrA, qnrB, qnrS, qepA, and aac(6´)-Ib-cr* genes were screened by PCR (Table 1). The *cr* variant has two amino acid changes, Trp-102-Arg and Asp-179-Try [14]. Amplifications were performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT, USA). PCR reagents were purchased from Promega (Madison, WI, USA).

Results and Discussion

The evolution of quinolone-resistant *Aeromonas* spp. was studied from 2000 to 2007 in an area of 286,774 inhabitants in Zaragoza, Spain. Of the *Aeromonas* strains isolated from stools samples, 129 (33.76%) were resistant to nalidixic acid, with the following yearly distribution over the study period: 2000 (11 strains, 40.7%), 2001 (15 strains, 35.7%), 2002 (9 strains, 25.7%), 2003 (31 strains, 37.3%), 2004 (17 strains, 29.31%), 2005 (14 strains, 30.43%), 2006 (25 strains, 52.1%), and 2007 (13 strains, 30.2%).

The pattern of *Aeromonas* spp. susceptibility to quinolones is shown in Table 2. Ciprofloxacin, norfloxacin, and ofloxacin susceptibility in the tested strains had a bimodal distribution, suggesting that more than one mechanism of resistance is involved in modulating the final MIC.

Among the quinolones evaluated, the rank order of intrinsic activity (MIC_{90}, mg/l) was: ciprofloxacin (4) < ofloxacin (8) < norfloxacin (32) < nalidixic acid (512), and the range of activity was <0.12–64 mg/l for ciprofloxacin, 0.5–64 mg/l for ofloxacin, 0.5–64 mg/l for norfloxacin, and 32–1024 mg/l for nalidixic acid.

Among the nalidixic-acid-resistant strains of *Aeromonas* spp. studied, 24 were identified as *A. veronii* bv. *sobria* and

Table 3. Number of *Aeromonas* isolates relative to a specific MIC (mg/l)

	MICs (mg/l) / No. of isolates																
	2048	1024	512	256	128	>64	64	32	16	8	4	2	1	0.5	0.25	0.12	<0.12
Nalidixic acid	0	1	6	17	12	–	1	3	np	np	np	np	np	np	np	np	np
Ciprofloxacin	np	np	np	np	np	1	1	0	0	0	2	12	12	5	2	1	4
Norfloxacin	np	np	np	np	np	2	0	2	11	8	3	8	4	2	0	0	0
Ofloxacin	np	np	np	np	np	2	0	0	0	5	16	14	2	1	0	0	0

(–) Higher dilutions of nalidixic acid were made; (np) not performed.

16 as *A. caviae*. While other authors have identified additional resistant *Aeromonas* species, the samples obtained in our study comprised only these two resistant species. *Aeromonas caviae* strains were more resistant to ciprofloxacin (18.75%), norfloxacin (43.75%), and ofloxacin (18.75%) than *A. veronii* bv. *sobria* (4%, 33.33%, and 8.3%, respectively). Both *A. caviae* and *A. veronii* bv. *sobria* had reduced susceptibility (intermediate) to the fluoroquinolones ciprofloxacin (12.5 and 37.5%, respectively), norfloxacin (12.5 and 25%, respectively), and ofloxacin (31.25 and 45.83%, respectively) (Table 3).

All 40 strains selected for QRDR sequencing in the *gyrA* and *parC* genes had only a single mutation in *gyrA*, at position 83. Sequence analysis of *gyrA* in 33 of the 40 strains studied showed the double base-pair change AGC → ATC at position 83, which resulted in the amino acid substitution Ser-83-Ile. In four other strains, the substitution was Ser-83-Val and in the remaining three strains Ser-83-Arg.

As previously described [9], mutations at residue 83 are the most frequently encountered and they confer the most significant increase in the level of quinolone resistance. In the isolates carrying the Ser-83-Val substitution, quinolone resistance is most likely explained by a two-step process: first, the mutation AGC → GGC (Ser → Ala), and later GGC → GTC (Ala → Val). A similar process is thought to underlie the low-level resistance to quinolones in other microorganisms, such as *E. coli* [27]. Substitutions involving amino acid 87, described as a second target, were not detected in any of our strains. Double mis-sense mutations in *gyrA* QRDR, particularly at positions 83 and 87, have been associated with an increase in fluoroquinolone resistance [20]. However, while we found double mutations at the same position (amino acid 83) in *gyrA* of our quinolone-resistant strains, none were identified at two different positions (amino acids 83 and 87) within the gene, consistent with in vitro findings in *E. coli* [27].

Alterations in the QRDR sequence of ParC were detected in 19 of the 40 nalidixic-acid-resistant strains, all of them consisting of a Ser-83-Ile substitution in *gyrA*. In 13 strains, the substitution was Ser-80-Ile. Seven of these strains had intermediate susceptibility to ciprofloxacin, five were susceptible, and one was resistant. In another five strains, Ser-80-Arg resulted in intermediate susceptibility to ciprofloxacin in two strains while the remaining three were susceptible. In one strain, with the substitution Ser-80-Thr, resistance to all studied fluoroquinolones was determined.

The different substitutions found in GyrA at Ser-83 and in ParC at Ser-80 were not predictive of either the susceptibility patterns or the MIC values, as shown in Table 3. The 33 strains with the Ser-83-Ile substitution in GyrA had a wide range of MICs for nalidixic acid (32–512 mg/l), without differences between strains carrying a single amino acid substitution and those also containing a *parC* mutation. Four strains with a Ser-83-Val substitution in GyrA and without any substitution in ParC had nalidixic acid MICs of 128 mg/l (1 strain), 256 mg/l (2 strains) and 512 mg/l (1 strain). Three strains with a Ser-83-Arg substitution in GyrA and without any substitutions in ParC had nalidixic acid MICs of 128 mg/l, 256 mg/l, and 1024 mg/l. For *Aeromonas* strains carrying a double *gyrA-parC* mutation, quinolone MICs were not higher than those for strains with a single *gyrA* mutation. Therefore, additional mechanisms probably account for the highest levels of quinolone resistance in these strains. In addition, our results point to heterogeneity in the *gyrA* and *parC* mutations with respect to nalidixic acid resistance, as previously demonstrated in *Y. enterocolitica* [3]. In other studies, double *gyrA-parC* mis-sense mutations in *Aeromonas* were shown to be associated with higher levels of quinolone resistance than was the case for single *gyrA* mutations [9].

Only one *Aeromonas* spp. strain, identified as *A. caviae*, carried the *qnrS2* gene. This strain also had a single mutation

Table 4. Mutations in *gyrA* and *parC* genes, QRDR substitutions, and quinolone susceptibilities

	GyrA and ParC substitutions				MICs (mg/l)			
Isolate	GyrA_83	Aa	ParC_80	Aa	Nalidixic acid	Ciprofloxacin	Norfloxacin	Ofloxacin
A. veronii	ATC	Ile	ATC	Ile	32	2	16	4
A. veronii	ATC	Ile	ATC	Ile	32	1	8	4
A. caviae	ATC	Ile	nm		32	1	2	2
A. veronii	ATC	Ile	AGA	Arg	64	1	8	2
A. veronii	ATC	Ile	ATC	Ile	128	2	16	4
A. caviae	ATC	Ile	ATC	Ile	128	4	16	4
A. veronii	GTC	Val	nm		128	1	2	2
A. veronii	ATC	Ile	nm		128	2	8	4
A. caviae	ATC	Ile	ATC	Ile	128	2	16	4
A. veronii	ATC	Ile	ATC	Ile	128	0.5	8	4
A. caviae	AGA	Arg	nm		128	0.25	2	1
A. caviae	ATC	Ile	ATC	Ile	128	2	32	8
A. veronii	ATC	Ile	nm		128	1	16	8
A. veronii	ATC	Ile	nm		128	0.12	1	2
A. veronii	ATC	Ile	nm		128	<0.12	0.5	2
A. veronii	ATC	Ile	nm		128	<0.12	0.5	2
A. caviae	GTC	Val	nm		256	1	16	8
A. veronii	AGA	Arg	nm		256	<0.12	1	0.5
A. veronii	ATC	Ile	nm		256	1	2	4
A. caviae	ATC	Ile	ATC	Ile	256	1	8	4
A. caviae	ATC	Ile	AGA	Arg	256	0.5	1	2
A. veronii	ATC	Ile	nm		256	0.5	1	2
A. veronii	ATC	Ile	ATC	Ile	256	1	16	4
A. veronii	ATC	Ile	ATC	Ile	256	2	32	4
A. veronii	ATC	Ile	AGA	Arg	256	1	8	4
A. veronii	GTC	Val	nm		256	2	4	2
A. caviae	ATC	Ile	ATC	Ile	256	2	8	4
A. veronii	ATC	Ile	nm		256	2	4	4
A. caviae	ATC	Ile	nm		256	0.5	2	8
A. veronii	ATC	Ile	AGA	Arg	256	2	16	2
A. veronii	ATC	Ile	AGA	Arg	256	2	16	8
A. veronii	ATC	Ile	ATC	Ile	256	<0.12	2	2
A. caviae	ATC	Ile	nm		256	1	4	4
A. caviae(*)	ATC(*)	Ile(*)	ACT(*)	Thr(*)	512(*)	>64(*)	>64(*)	>64(*)
A. caviae	ATC	Ile	nm		512	1	16	4
A. caviae	ATC	Ile	nm		512	0.25	2	2
A. veronii	GTC	Val	nm		512	4	8	2
A. caviae	ATC	Ile	nm		512	0.5	2	1
A. veronii	ATC	Ile	ATC	Ile	512	2	16	2
A. caviae	AGA	Arg	nm		1024	64	>64	>64

(*) The isolate harboring the *qnrS2* gene. (nm) No mutation.

in *gyrA* at position 83 (Ser-83-Ile) and a mutation in *parC* (Ser-80-Thr) (previously characterized) [1]. Neither the *qnrA* nor the *qnrB* gene was detected in any of the 40 strains. Two isolates were positive for *aac(6´)-Ib*, but no *-cr* variants were found. Plasmid-borne *qnrS* determinants in non-enterobacterial species have not been well-studied, although they reportedly have been detected in two *Aeromonas punctata* ssp. *punctata* and *A. media* isolates from the Seine River in Paris [4], a single *Aeromonas allosaccharophila* isolate from Lake Lugano in Switzerland [22], and a clinical isolate of *A. veronii* [16].

All of the 24 *A. veronii* bv. *sobria* strains had only one substitution in GyrA QRDR, all resulting in an amino acid change at Ser-83. Sequence analysis of *gyrA* showed a Ser-83-Ile substitution in 20 of the 24 strains, a Ser-83-Val substitution in three strains, and a Ser-83-Arg substitution in one strain. Among the strains with a Ser-83-Ile substitution in *gyrA*, 12 had also a substitution in the ParC QRDR: in eight strains a Ser-80-Ile substitution and in four strains a Ser-80-Arg substitution. Of the 16 *A. caviae* strains studied, all had only a single mutation at position 83 of *gyrA*: in 13 strains Ser-83-Ile, in two strains Ser-83-Arg, and in one strain Ser-83-Val. Of these strains, all those with a Ser-83-Ile substitution in *gyrA* also had a substitution in ParC QRDR: in five strains Ser-80-Ile, in one strain Ser-80-Arg, and in one strain Ser-80-Thr. This observation strongly suggests that in mesophilic *Aeromonas* strains, as in other gram-negative bacteria, DNA gyrase is the primary target of quinolones [17], since all quinolone-resistant strains carried a Ser substitution at position 83, with a mutation to Ile being the most frequent (Table 4).

In the presence of Phe-Arg-β-naphthylamide 20 mg/l, the MICs of nalidixic acid were reduced two- to six-fold while those of ciprofloxacin were not affected. These results support an efflux-mediated resistance mechanism in the development of high levels of resistance to nalidixic acid, as previously described for *Y. enterocolitica* [3], *E. coli* [21], and *S. maltophilia* [18]. In addition, our data suggest that quinolone resistance in *Aeromonas* isolated from patients with diarrhea is due to chromosomal mutations in *gyrA*. The same resistance mechanism has been found in environmental *Aeromonas* isolates [9]. Nevertheless, detection of the plasmid-mediated quinolone resistance determinant *qnrS* in a clinical isolate with a high level of ciprofloxacin resistance should increase our awareness of the potential for the widespread dissemination of this resistance mechanism. Moreover, it emphasizes the necessity to analyze all resistant strains at the molecular level in order to identify new patterns of resistance.

Although the focus of this study was clinical isolates, members of the genus *Aeromonas* are ubiquitous in aquatic ecosystems worldwide, including groundwater and drinking-water treatment plants. Quinolones are synthetic antibiotics that normally should be absent from fresh water. Therefore, as suggested by Goñi et al. [9], the discharge of quinolones, probably from agricultural sources, into rivers may promote the selection of resistant mutants among indigenous bacterial populations. Indeed, quinolones, which are widely used in veterinary medicine, are mostly excreted unchanged and they are among the most persistent drugs in the environment. Mesophilic aeromonads, particulary those associated with human infections, have been found in a wide variety of fresh produce, meat (beef, poultry, pork), and dairy products (cow milk, ice cream). Therefore, the selective pressure of antimicrobial agents in both veterinary and human clinical environments may allow the survival of these bacteria, with resistance determinants in strains infecting food-producing animals transmitted throughout the food chain. An efficient surveillance for quinolone resistance in *Aeromonas* species should include the study of isolates from human, animal, food, and drinking-water origins.

Although fluoroquinolones have been reported as the treatment of choice for *Aeromonas* infections, it is well-established that nalidixic acid resistance predicts the development of fluoroquinolone resistance during therapy, as well as therapeutic failure [14]. In order to avoid inadequate empirical antibiotic treatment, the appearance of quinolone resistance in other geographical areas should be monitored through continuous surveillance studies. This strategy should include epidemiological surveys to evaluate the prevalence of chromosomally encoded mutations and the emergence of plasmid-mediated quinolone resistance among *Aeromonas* species identified in clinical isolates, such as *A. hydrophila*, *A. caviae*, and *A. veronii*.

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