Letter to the Editor

Plasmid-mediated quinolone resistance determinant *qnrB19* in non-typhoidal *Salmonella enterica* strains isolated in Venezuela

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Quinolone resistance in *Enterobacteriaceae* is typically mediated by chromosomal mutations leading to alterations in the target enzymes DNA gyrase and topoisomerase IV, or changes in drug entry and efflux. However, three plasmid-mediated quinolone resistance (PMQR) mechanisms conferring decreased susceptibility to quinolones (including some fluoroquinolones) have been recently described: QepA and OqxAB effluxes, Aac(6′)-Ib-cr aminoglycoside acetyltransferase and Qnr proteins (*qnrA, qnrB, qnrC, qnrD* and *qnrS*) [1,2]. Although plasmid-mediated quinolone resistance of Qnr type has been identified in *Enterobacteriaceae* from the United States, Europe, and Asia [1,2], little is known about the diversity, type or range of *qnr* genes in Latin America [3-6], especially in Venezuela. Therefore, the objective of this study was to screen for the presence of PMQR genes in non-typhoidal *Salmonella enterica* (NTS) strains with reduced susceptibility to fluoroquinolones, from clinical samples and chicken meat in Venezuela.

A total of 127 NTS strains belonging to the collection of the Molecular Microbiology Laboratory of the Pharmacy Faculty of University of the Andes (Mérida, Venezuela), were enrolled in this study. These included 117 strains from stool specimens of pediatric patients collected from 2005 to 2007 and 10 isolates recovered from raw chicken meat in 2008. Six (4.7%) of these isolates showed a typical phenotype with reduced resistance to ciprofloxacin (MICs: 0.5 – 1 μg/mL) and with affiliated susceptibility to nalidixic acid (MICs: 4 - 8 μg/mL). These isolates were screened for extended-spectrum beta-lactamase (ESBL) phenotype, using cefotaxime and ceftazidime with and without clavulanic acid per Clinical and Laboratory Standards Institute (CLSI) guidelines [7] and confirmed by polymerase chain reaction (PCR) using specific primers for bla_{TEM}, bla_{SHV} and group $bla_{\text{CTX-M}}$ [8]. Presence of *qnrA, qnrB, qnrS, qnrD, aac(6′)-Ib* and *qepA* genes was screened by multiplex and simplex PCR amplifications, using primers and conditions previously described [9,10]. In addition, mutations in quinolone resistance-determining regions (QRDR) of the *gyrA*, *gyrB* and *parC* genes were also determined [11]. Amplicons were sequenced to determine the gene variants and mutations.

Regardless of origin and serovar, the *qnrB* gene was detected in six *Salmonella* strains. Sequence analysis of the amplification product revealed the *qnrB19* variant (Table). No mutations were identified in the QRDR of the *gyrA*, *gyrB* and *parC* genes [11]. In four of these strains the presence of ESBLs was suspected from ceftazidime or cefotaxime resistance (MICs: 64 – >256 μg/mL) and by reestablishing the susceptibility in the presence of clavulanic acid (4 μg/mL) [7]. PCR amplification, using specific primers for bla_{TEM}, bla_{SHV} and group $bla_{\text{CTX-M}}$ [11], followed by sequencing analysis, allowed us to identify $bla_{\text{TEM-1}}$ + $bla_{\text{SHV-12}}$ in *S*. Give LMM96 and

Table. Characteristics of *Salmonella* serovar isolates harboring *qnr* and β-lactamase genes

Isolate Number	Year of collection	Serovar	Sample	MIC μg/mL					*qnr* gene	β-lactamase	PFGE Profile
				CIP	NAL	CTX	CAZ	CTX/CLA			
LMM46	2006	Havana	Human	1	4	0.25	2	-	*qnrB*19	-	C
LMM96	2006	Give	Human	1	8	4	32	0.125	*qnrB*19	$bla_{\text{TEM-1}}$ $bla_{\text{SHV-12}}$	A1
LMM183	2007	Give	Human	1	4	128	32	0.25	*qnrB*19	$bla_{\text{TEM-1}}$ $bla_{\text{CTXM-2}}$	A2
LMM175	2008	Heidelberg	Chicken	0.5	8	>256	32	1	*qnrB*19	$bla_{\text{TEM-1}}$ $bla_{\text{CTXM-2}}$	B1
LMM179	2008	Heidelberg	Chicken	1	4	>256	32	0.5	*qnrB*19	$bla_{\text{TEM-1}}$ $bla_{\text{CTXM-2}}$	B2
LMM300	2008	Meleagridis	Chicken	0.5	4	0.25	1	-	*qnrB*19	-	D

MIC: minimal inhibitory concentration; CIP: ciprofloxacin; NAL: nalidixic acid; CTX: cefotaxime; CAZ: ceftazidime; CTX/CLA: cefotaxime/clavulanic acid.

$bla_{\text{TEM-1}}$ + $bla_{\text{CTX-M-2}}$ in the other three strains: *S*. Give LMM183, *S*. Heidelberg LMM175 and *S*. Heidelberg LMM179 (Table). Association between QnrB-like determinants and ESBLs has been previously reported [2-4,6].

Qnr-positive plasmids were successfully transferred by transformation from serovars *Salmonella* to *E. coli* HB101. Transformants designated as LMM46-T, LMM96-T, LMM183-T, LMM175-T, LMM179-T and LMM300-T, could be selected on Mueller Hinton Agar plates supplemented with 0.06 μg/ml ciprofloxacin. PCR and sequencing confirmed the presence of *qnrB19* in the transformants obtained. Susceptibility testing showed that the MICs of ciprofloxacin and nalidixic acid for all transformants were similar to corresponding host strains. Plasmid DNA was purified from transformants by an alkaline lysis method [12]. Electrophoresis showed the presence of a ~17 kb plasmid with a similar restriction pattern, using the PstI enzyme (Promega, Madison, WI, USA) in all the transformants obtained.

All *qnr*-positive isolates were typed using PFGE [13]. In total, four PFGE clusters were identified (A-D) and isolates of different serotypes were clustered separately. Similar distribution patterns have also been observed in NTS isolates with reduced susceptibility to ciprofloxacin from infants in Wuhan, China [14].

This result indicates that the horizontal transfer PMQR occurs, since the same plasmid profile was observed in strains from different origins, times, and serovars. Fluoroquinolones are widely used in veterinary medicine as well as in poultry production, and *qnr*-positive NTS isolates could be selected and transmitted to humans through the food chain.

As far as the authors know, this is the first description of the occurrence of the *qnrB19* gene in NTS isolates from pediatric patients and chicken meat in Venezuela. Isolates similar to those described in this study may be hard to identify in clinical laboratories since this phenotype is difficult to recognize by conventional methods. In addition, the *qnrB19* gene found in enteropathogens, classified as susceptible to fluoroquinolones, may promote further selection from low- to high-level resistance when fluoroquinolones are used. Hence it is necessary to increase the sensitivity and optimize the screening procedures when strains that might contain such resistance determinants are studied.

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