RESEARCH LETTER

Characterization of antimicrobial resistance in *Salmonella enterica* food and animal isolates from Colombia: identification of a *qnrB19*-mediated quinolone resistance marker in two novel serovars

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Abstract

Ninety-three *Salmonella* isolates recovered from commercial foods and exotic animals in Colombia were studied. The serotypes, resistance profiles and where applicable the quinolone resistance genes were determined. *Salmonella* Anatum ($n = 14$), Uganda (19), Braenderup (10) and Newport (10) were the most prevalent serovars, and resistance to tetracycline (18.3%), ampicillin (17.2%) and nalidixic acid (14%) was most common. Nalidixic acid-resistant isolates displayed minimum inhibitory concentrations ranging from 32 to 1024 $\mu g\,mL^{-1}$. A Thr57 → Ser substitution in ParC was the most frequent (12 of the 13 isolates). Six isolates possessed an Asp87 → Tyr substitution in GyrA. No alterations in GyrA in a further seven nalidixic acid-resistant isolates were observed. Of these, four serovars including two Uganda, one Infantis and a serovar designated 6,7:d:-, all carried *qnrB19* genes associated with 2.7 kb plasmids, two of which were completely sequenced. These exhibited 97% (serovar 6,7:d:- isolate) and 100% (serovar Infantis isolate) nucleotide sequence identity with previously identified ColE-like plasmids. This study demonstrates the occurrence of the *qnrB19* gene associated with small ColE plasmids hitherto unrecognized in various *Salmonella* serovars in Colombia. We also report unusual high-level quinolone resistance in the absence of any DNA gyrase mutations in serovars *S.* Carrau, Muenchen and Uganda.

Introduction

Salmonellosis is a classic food-borne infection that constitutes a major public health problem. Contaminated food of animal origin including eggs, meat, unpasteurized dairy products, seafood, fruits and vegetables are sources of infections with *Salmonella* spp. (Mead *et al.*, 1999).

Antimicrobial-resistant salmonellae constitute a health hazard due to the increased risk of therapeutic failure in cases where chemotherapy is indicated. Fluoroquinolones are the drugs of choice to treat invasive, life-threatening salmonellosis. In these zoonotic pathogens, the emergence of fluoroquinolone resistance or reduced susceptibility is particularly challenging (Tollefson *et al.*, 1997; Dimitrov *et al.*, 2007).

Quinolone resistance in *Salmonella* spp. is principally caused by mutations in the target enzymes, DNA gyrase and topoisomerase IV (Griggs *et al.*, 1996; Piddock *et al.* 1998; Piddock, 2002; Eaves *et al.*, 2004). Other mechanisms such as increased activity of efflux pumps, decreased permeability due to loss of porins and a variety of plasmid-mediated quinolone resistance (PMQR) mechanisms also contribute to resistance and/or decreased susceptibility, one of the latter being the *qnr* gene (Martínez-Martínez *et al.*, 1998; Piddock, 2002; Robicsek *et al.*, 2005; Giraud *et al.*, 2006; Strahilevitz *et al.*, 2009).

Rapid dissemination of plasmid-mediated *qnr* genes has been described in recent years (Robicsek *et al.*, 2006; Cattoir *et al.*, 2007; Hopkins *et al.*, 2007; Minarini *et al.*, 2008; Wu *et al.*, 2008; Cerquetti *et al.*, 2009; Cui *et al.*, 2009;

García-Fernández *et al*., 2009; Gunell *et al*., 2009). Qnr proteins share common structural properties and belong to a pentapeptide family of proteins. By virtue of their capacity to bind specifically to DNA gyrase, these proteins limit access of the fluoroquinolone drug to its target, thereby providing protection to the bacteria (Tran *et al*., 2005). Five different *qnr* genes have been described: *qnrA*, *B*, *C*, *D* and *S* with a number of variants exhibiting minor sequence differences (Martínez-Martínez *et al*., 1998; Hata *et al*., 2005; Jacoby *et al*., 2006; Cavaco *et al*., 2009; Wang *et al*., 2009). The first *qnrB* gene described was reported in a *Klebsiella pneumoniae* isolate from India and was located on a plasmid carrying the $bla_{CTX-M-15}$-mediated ESL resistance marker (Jacoby *et al*., 2006). Qnr proteins have been identified in both clinically resistant and susceptible isolates. The minimum inhibitory concentrations (MICs) for nalidixic acid and ciprofloxacin reported in these isolates ranged from twofold to eightfold and 8–32-fold higher, respectively, when compared with the isogenic progenitor isolates (Jacoby *et al*., 2006; Minarini *et al*., 2008; Murray *et al*., 2008; Strahilevitz *et al*., 2009).

Recently, *qnrB* determinants were found ubiquitous in commensal microbial communities of healthy children in Peru and Bolivia and were subsequently found to be encoded by small ColE-type plasmids (Pallecchi *et al*., 2009, 2010).

In this paper, we report on a study of 93 *Salmonella* isolates recovered from foods and exotic animals in Colombia. Serotyping was used to initially characterize the isolates, and their resistance profiles were determined. A plasmid-mediated *qnrB19* marker was detected in four isolates and this gene was completely characterized.

Materials and methods

Bacterial isolates

A collection of 93 *Salmonella* spp. isolates recovered between 2002 and 2009 from a variety of food products and animals in Colombia was obtained from the University of Cordoba (Colombia). Isolates were streaked on XLD medium (Oxoid, Basingstoke, UK) to check for purity, and were confirmed as *Salmonella* using a *Salmonella* latex test (Oxoid).

Drug susceptibility testing

Susceptibilities to 15 drugs were determined by disc diffusion and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2007). The following antimicrobial compounds were used: amoxicillin–clavulanic acid 20/10 μg (AMC), ampicillin 10 μg (AMP), cefpirome 30 μg (CFP), cefpodoxime 10 μg (CPD), ceftiofur 30 μg (CFR), cephalothin 30 μg (KF), chloramphenicol 30 μg (C), ciprofloxacin 5 μg (CIP), gentamicin 10 μg (GM), kanamycin 30 μg (KAN), nalidixic acid 30 μg (NA), neomycin 30 μg (NEO), streptomycin 10 μg (S), trimethoprim/sulfamethoxazole 25 μg (SXT), and tetracycline 30 μg (TE). Discs were purchased from Oxoid. *Escherichia coli* ATCC® 25922 was included as a control.

MIC determination

MICs for nalidixic acid (Sigma-Aldrich, Ireland) and ciprofloxacin (Sigma-Aldrich) were determined by the broth microdilution method (CLSI, 2007), in the absence and presence of 40 μg mL^{-1} phe-arg-β-naphthylamide (PAβN) (Sigma–Aldrich).

Nucleic acid purification and DNA sequencing

Genomic DNA extraction, PCR purification and sequencing were performed as described previously (O'Regan *et al*., 2009). Table 1 provides the details of all primer sequences, annealing temperatures and amplicon sizes. Positive controls for the detection of PMQR genes were included: *E. coli Lo qnrA1*+, *K. pneumoniae* B1 *qnrB1*+, *E. coli* S7 *qnrS1*+, *E. coli* TOP10+pCR2.1W *qepA* and *E. coli* 78-01 *aac(6′)-Ib-cr*+.

Nalidixic acid-resistant isolates were assessed for all known PMQR markers using previously published primers (Table 1). Plasmids were purified from nalidixic acid-resistant isolates using the PureYield™ Plasmid Midiprep System (Promega, Madison, WI) and their profiles were determined in a 0.9% agarose gel SeaKem®LE Agarose (Lonza, Wokingham, UK) after electrophoresis in 1 × Tris-HCl (pH 8)–boric acid–EDTA buffer containing 0.1 μg mL^{-1} ethidium bromide (Sigma-Aldrich). Using a PCR-based method developed previously by Pallecchi *et al*. (2010), the ColE-like plasmid carrying *qnrB19* genetic determinant was amplified and the sequence was determined (Qiagen, Hilden, Germany). Complete amplified plasmid products were subjected to restriction fragment length polymorphism (RFLP) analysis with MboII enzyme (New England Biolabs, Ipswich, MA) to identify any sequence-based polymorphisms.

The complete sequence of these plasmids was determined (Qiagen) and analysed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/), CLUSTALW (http://www.ebi.ac.uk/clustalw/) and DNASTAR (DNAStar Inc., Madison, WI) programs.

Nucleotide sequence accession numbers

Nucleotide sequences determined were deposited in GenBank under accession numbers HM070379 and HM070380.

Results and discussion

Serotypes

Table 2 shows the serovars recovered, along with their source and geographical origin, date of isolation and corresponding susceptibility patterns. In all, 19 serovars were identified,

Table 1. Target genes, amplification primers and PCR reaction conditions used for characterization of the collection

Target	Primer	Sequence (5′-3′)	Annealing temperature (°C)	Product size (bp)	Reference
Sequencing primers					
gyrA	GyrA_For	TGT CCG AGA TGG CCT GAA GC	55	470	Modified from Carrique-Mas *et al*. (2008)
	GyrA_Rev	CGT TGA TGC TTC CGT CAG			
gyrB	GyrB_For	GAA ATG ACC CGT CGT AAA GG	54	710	O'Regan *et al*. (2009)
	GyrB_Rev	TAC AGT CTG CTC ATC AGA AAG			
parC	ParC_For	ATG AGC GAT ATG GCA GAG CG	52	413	Carrique-Mas *et al*. (20080
	ParC_Rev	TGA CCG AGT TCG CTT AAC AG			
parE	ParE_For	GAC CGA GCT GTT CCT TGT GG	52	493	Carrique-Mas *et al*. (2008)
	ParE_Rev	GCG TAA CTG CAT CGG GTT CA			
qnrB	qnrB-CS-2A	GTT GGC GAA AAA ATT GAC AGA A	52	500	Wu *et al*. (2008)
	qnrB-CS-3B	ACTCCGAATTGGTCAGATCG			
qnrB19^{+} plasmid	qnrB19_FW	TGG ATG GGG ACT CAG GTA CT	55	2700	Pallecchi *et al*. (2010)
	qnrB19_RV	CGG CAC CTG AAA AAT CGC AG			
Detection primers					
qnrA1 to *qnrA6*	QnrAm-F	AGA GGA TTT CTC ACG CCA GG	54	580	Cattoir *et al*. (2007)
	QnrAm-R	TGC CAG GCA CAG ATC TTG AC			
qnrB1 to *qnrB6*	QnrBm-F	GGM ATH GAA ATT CGC CAC TG	54	264	Cattoir *et al*. (2007)
	QnrBm-R	TTT GCY GYY CGC CAG TCG AA			
qnrS1 to *qnrS2*	QnrSm-F	GCA AGT TCA TTG AAC AGG GT	54	428	Cattoir *et al*. (2007)
	QnrSm-R	TCT AAA CCG TCG AGT TCG GCG			
qepA	QEPfor	TGG TCT ACG CCA TGG ACC TCA	56	1137	M. Galimand, pers. commun.
	QEPrev	TGA ATT CGG ACA CCG TCT CCG			
aac(6′)-Ib	aac(6′)-Ib_For	TTG CGA TGC TCT ATG AGT GGC TA	55	482	Park *et al*. (2006)
	aac(6′)-Ib_Rev	CTC GAA TGC CTG GCG TGT TT			
ColE-like plasmid backbone	ColEPB_FW	CTG ACA CTC AGT TCC GCG A	55	900	Pallecchi *et al*. (2010)
	qnrB19ColEPB_RV	CGG CAC CTG AAA AAT CGC AG			

with *S*. Uganda ($n = 19$), Anatum ($n = 14$), Braenderup ($n = 10$) and Newport ($n = 10$) predominating, followed by serovars Carrau ($n = 8$), Infantis ($n = 7$), Saintpaul ($n = 5$), Muenchen ($n = 4$) and Rubislaw ($n = 3$). Fresno, Javiana and Senftenberg serovars were represented by two isolates each. Single isolates belonging to serovars Adelaide, Bredeney, Derby, Gaminara, *Salmonella enterica* ssp. *enterica* 6,7:d:-, Minnesota, and Typhimurium, were also identified in this collection. No Enteritidis serovars were recovered. The most common serovars implicated in human salmonellosis in Colombia are Enteritidis and Typhimurium (Munoz *et al*., 2006; Wiesner *et al*., 2006). However, serovars identified in this study have occasionally been implicated in salmonellosis outbreaks worldwide (Lehmacher *et al*., 1995; Jones *et al*., 2004; Gupta *et al*., 2007; Lang, 2008).

Antimicrobial susceptibility profiles

A summary of the resistance profiles obtained for each isolate against a panel of 15 antimicrobial compounds is shown in Table 3. Forty-six percent ($n = 40$) were resistant to at least one antimicrobial agent. Tetracycline resistance was the most common resistance property encountered (18.3%, $n = 17$), followed by ampicillin resistance (17.2%; $n = 16$), and nalidixic acid resistance (14%; $n = 13$). Multidrug-resistant isolates (defined as resistant to three or more different drug classes) constituted 4.3% of the collection ($n = 4$).

The emergence of quinolone resistance together with reduced ciprofloxacin susceptibility in *S. enterica* is increasingly observed and constitutes a major concern because infections with such isolates may cause ciprofloxacin treatment failure (Dimitrov *et al*., 2007). While the frequency of quinolone resistance in *Salmonella* is growing worldwide, in this study, 14% of the isolates were resistant to nalidixic acid, a figure that could be considered high (Marimón *et al*., 2004; Stevenson *et al*., 2007). This corresponded to the data in the SENTRY Antimicrobial Surveillance program, which reported nalidixic acid resistance of 14% in *Salmonella* spp. isolates from Latin America during the years 1997–2004, a figure more than twofold higher than that recorded in North America (Biedenbach *et al*., 2006).

MIC testing

In the case of the isolates showing resistance to quinolone-based antimicrobial compounds, an MIC for nalidixic acid of 32 μg mL^{-1} was recorded for two isolates, 256 μg mL^{-1} for three, and 1024 μg mL^{-1} for eight isolates.

Table 2. Characterization of 93 *Salmonella enterica* isolates from Colombia

Isolate no.	Date of isolation	Source	Geographical area	Serovar	Resistance profile
S1	23/11/2002	Ham	Barranquilla	Carrau	–
S2	14/03/2003	Ham	Barranquilla	Carrau	–
S3	20/10/2004	Cheese	Sincelejo	Anatum	AMP, AMC, KF, TE
S4	20/10/2004	Retail chicken	Sincelejo	Infantis	–
S5	20/10/2004	Cheese	Sincelejo	Infantis	–
S6	28/05/2004	Meat*	Sincelejo	Newport	–
S7	28/05/2004	Retail chicken	Sincelejo	Newport	–
S8	28/05/2004	Sausages	Sincelejo	Newport	–
S9	28/05/2004	Cheese	Sincelejo	Infantis	S
S10	22/09/2004	Retail chicken	Cartagena	Uganda	S
S11	22/09/2004	Retail chicken	Cartagena	Uganda	–
S12	22/09/2004	Meat	Cartagena	Uganda	–
S13	22/09/2004	Retail chicken	Cartagena	Uganda	–
S14	22/09/2004	Meat	Cartagena	Uganda	–
S15	27/10/2004	Ground meat	Sincelejo	Newport	–
S16	06/12/2004	Ham	Montería	Newport	–
S17	06/12/2004	Ham	Montería	Newport	–
S18	06/12/2004	Retail chicken	Montería	Newport	–
S19	06/12/2004	Retail chicken	Montería	Braenderup	TE
S20	07/12/2004	Retail chicken	Sincelejo	Infantis	KAN, NEO, NA, S, TE
S21	03/01/2005	Ham	Barranquilla	Minnesota	–
S22	03/01/2005	Intestine bovine	Barranquilla	Adelaide	–
S23	03/01/2005	Salty meat	Barranquilla	Newport	–
S24	01/02/2005	Sausages	Monteria	Uganda	KAN, NEO, NA, S, TE
S25	01/02/2005	Pig	Monteria	Newport	–
S26	01/02/2005	Retail chicken	Monteria	Infantis	–
S27	01/02/2005	Ham	Monteria	Anatum	–
S28	28/03/2005	Cheese	Cartagena	Anatum	S, TE
S29	28/03/2005	Meat	Cartagena	Uganda	–
S30	28/03/2005	Intestine bovine	Cartagena	Braenderup	–
S31	28/03/2005	Meat	Cartagena	Saintpaul	–
S32	28/03/2005	Bovine spleen	Cartagena	Saintpaul	–
S33	12/04/2005	Sausages	Monteria	Anatum	–
S34	12/04/2005	Ground meat	Monteria	Rubislaw	–
S35	12/04/2005	Ground meat	Monteria	Rubislaw	–
S36	12/04/2005	Corn and egg mixture	Monteria	Rubislaw	–
S37	07/06/2005	Potato	Cartagena	Muenchen	NA
S38	07/06/2005	Ground meat	Cartagena	Uganda	KAN, NEO, NA, S, TE
S39	07/06/2005	Ground meat	Cartagena	Uganda	KAN, NEO, TE
S40	07/06/2005	Meat	Cartagena	Uganda	–
S41	07/06/2005	Sausages	Cartagena	Carrau	–
S42	22/07/2005	Amazona spp. (parrot)	Monteria	Anatum	–
S43	16/08/2005	Cheese	Cartagena	Anatum	–
S44	16/08/2005	Ground meat	Cartagena	Anatum	NA
S45	16/08/2005	Meat	Cartagena	Carrau	NA
S46	16/08/2005	Cheese	Cartagena	Carrau	NA
S47	16/08/2005	Ground meat	Cartagena	Uganda	NA, TE
S48	10/10/2007	Sausages	Sincelejo	Anatum	TE
S49	10/10/2007	Sausages	Sincelejo	Saintpaul	–
S50	10/10/2007	Cheese	Sincelejo	Anatum	–
S51	10/10/2007	Cheese	Sincelejo	Carrau	NA
S52	18/10/2005	Ground meat	Cartagena	Carrau	NA, TE,
S53	18/10/2005	Ham	Cartagena	Anatum	NA
S54	18/10/2005	Cheese	Cartagena	Anatum	–
S55	18/10/2005	Sausages	Cartagena	Anatum	GM, S, TE
S56	18/10/2005	Ground meat	Cartagena	Anatum	–
S57	18/10/2005	Ham	Cartagena	Anatum	–

Table 2. Continued.

Isolate no.	Date of isolation	Source	Geographical area	Serovar	Resistance profile
S58	18/10/2005	Cheese	Cartagena	Muenchen	TE
S59	18/10/2005	Sausages	Cartagena	Uganda	TE
S60	18/10/2005	Meat	Cartagena	Saintpaul	AMP
S61	18/10/2005	Cheese	Cartagena	Muenchen	AMP
S62	04/09/2006	Ham	Monteria	Braenderup	AMP
S63	04/09/2006	Ground meat	Monteria	Braenderup	AMP
S64	14/10/2006	Cheese	Monteria	Muenchen	NA, TE
S65	25/02/2007	Salty meat	Monteria	Uganda	–
S66	25/02/2007	Salty meat	Monteria	Uganda	–
S67	25/02/2007	Ham	Monteria	Derby	TE
S68	25/02/2007	Sausages	Monteria	Uganda	–
S69	25/02/2007	Sausages	Monteria	Braenderup	TE
S70	25/02/2007	Sausages	Monteria	Uganda	–
S71	10/04/2007	Flour mandioka powder	Monteria	Braenderup	–
S72	10/04/2007	Flour mandioka powder	Monteria	Uganda	–
S73	16/04/2007	Ground meat	Monteria	Uganda	AMP
S74	16/04/2007	Meat	Monteria	Bredeney	AMP
S75	16/04/2007	Ground meat	Monteria	6,7:d:-	AMP, NA
S76	16/04/2007	Cheese	Monteria	Senftenberg	–
S77	16/04/2007	Cheese	Monteria	Braenderup	–
S78	16/04/2007	Ground meat	Monteria	Saintpaul	–
S79	24/04/2007	Meat	Monteria	Braenderup	KF
S80	24/04/2007	Ground meat	Monteria	Uganda	–
S81	24/04/2007	Ground meat	Monteria	Senftenberg	AMP
S82	24/04/2007	Sausages	Monteria	Braenderup	AMP
S83	24/04/2007	Sausages	Monteria	Javiana	AMP
S84	24/04/2007	Sausages	Monteria	Fresno	AMP
S85	04/05/2007	Sausages	Monteria	Infantis	AMP
S86	04/05/2007	Ground meat	Monteria	Gaminara	AMP
S87	04/05/2007	Potato and meat	Monteria	Infantis	AMP
S88	04/06/2007	*Iguana iguana*	Monteria	Braenderup	AMP
S91	26/05/2009	*Hydrochoerus hydrochaeris* (capybara)	Monteria	Fresno	C, KF, TE
S92	26/05/2009	*Hydrochoerus hydrochaeris*	Monteria	Typhimurium	AMC, KF
S93	28/05/2009	*Trachemys scripta callirostris* (hicotea)	Monteria	Javiana	–
S95	28/05/2009	*Trachemys scripta callirostris*	Monteria	Newport	–
S98	23/11/2002	Ham	Barranquilla	Carrau	–

*All meat belonged to bovines.

–, No resistances detected; AMP, ampicillin; AMC, amoxicillin–clavulanic acid; C, chloramphenicol; GM, gentamicin; KAN, kanamycin; KF, cephalothin; NA, nalidixic acid; NEO, neomycin; S, streptomycin; TE, tetracycline.

Reduced susceptibility to ciprofloxacin was noted for all 13 isolates (ranging from 0.5 to 1 $\mu g\,mL^{-1}$). A summary of the MIC data is presented in Table 4.

A 2–16-fold decrease in the MIC of nalidixic acid was observed In the presence of PAβN, a known efflux pump inhibitor (Table 4) with six isolates showing a 4–16-fold decrease. These results indicate that efflux pump activity may be contributing to the resistant phenotype and will be investigated in a separate follow-up study.

Target gene mutations

Target gene mutations associated with resistance to fluoroquinolones/quinolones (F)Q are shown in Table 4. One of the isolates did not possess any target gene mutations. Others possessed up to three mutations in the corresponding target genes. Six of 13 nalidixic acid-resistant isolates had mutations in the QRDR region of *gyrA;* in all these cases the Asp87 → Tyr substitution was noted. No amino acid sequence changes were identified in GyrB. Substitutions in ParC (Thr57 → Ser) were noted in 12 isolates. One had a Gly25 → Ala along with a second substitution within ParC (isolate S47, Table 4). Two different ParE mutations were identified: Asn446 → Pro in one isolate (S46) and Arg508 → Lys in another two isolates (S52 and S53, Table 4).

High-level resistance to nalidixic acid and decreased susceptibility to ciprofloxacin was observed in isolates S44, S45, S46, S51, S53 and S64, which could be attributed to the

single substitution in the GyrA previously found to correlate with this phenotype (Walker *et al.*, 2001; Eaves *et al.*, 2002; Ling *et al.*, 2003; Stevenson *et al.*, 2007). In isolates S20, S24, S38 and S75, nalidixic acid resistance could be attributed to the presence of PMQR. Characteristically, nalidixic acid MICs in these latter isolates were lower (ranging from 32 to 256 $\mu g\,mL^{-1}$) compared with isolates with the more common *gyrA* mutation. However, three remaining isolates of serovars Muenchen (denoted as S37), Uganda (S47) and Carrau (S52) did not possess GyrA substitutions, but were highly resistant to nalidixic acid (MIC = 1.024 $\mu g\,mL^{-1}$) and displayed reduced susceptibility to ciprofloxacin (MIC = 0.5–1 $\mu g\,mL^{-1}$). All three possessed the Thr57 → Ser ParC substitution. *Salmonella* Uganda (S47) also contained a second ParC amino acid change (Gly25 → Ala), and the Carrau isolate (S52) had an additional Arg508 → Lys substitution in ParE. Because these isolates possessed different mutations, it was difficult to conclude as to which mechanism was primarily responsible for the phenotype observed. Contribution of increased efflux activity is likely in the *S.* Muenchen and Uganda isolates as demonstrated by the MIC assay in the presence of PAβN. Nonetheless, MICs decreased to 128 and 256 $\mu g\,mL^{-1}$ in these two isolates, respectively, values that are indicative of clinical resistance, strongly suggesting the presence of (an) additional undefined mechanism(s).

Some reports suggest that the distribution of specific substitutions within target genes might differ depending on the serovar. Furthermore, the frequency with which these mutations are observed may reflect the impact of exposure to different fluoroquinolone drugs (Giraud *et al.*, 1999; Levy *et al.*, 2004). Nonetheless, mutation patterns in the isolates studied could not be correlated with specific serovars. The spectrum of mutations observed was narrow and only one polymorphism occurred in *gyrA*, suggesting that conditions under which the mutations developed might have been a common factor leading to this particular alteration.

The lack of *gyrA* mutations in some isolates together with the presence of *parC* mutations in six other isolates is a unique finding. Although the Thr57 → Ser substitution in ParC has been reported previously in *Salmonella*, it is detected less frequently compared with the more common *gyrA* mutations and typically occurs concomitantly with double *gyrA* mutations (Piddock *et al.* 1998; Baucheron *et al.*, 2005; Hopkins *et al.*, 2005). The Thr57 → Ser mutation in *parC* was first reported by Ling *et al.* (2003) in *Salmonella* isolates with a wild-type DNA gyrase and others possessing single *gyrA* mutations, wherein the first were

Table 3. Prevalence of antibiotic resistance in the strain collection

Antibiotic	% Resistance (*n*)
Amoxicillin–clavulanic acid	2.15% (2)
Ampicillin	17.2% (16)
Cefpirome	0
Cefpodoxime	0
Ceftiofur	0
Cephalothin	4.3% (4)
Chloramphenicol	1.1% (1)
Ciprofloxacin	0
Gentamicin	1.1% (1)
Kanamycin	4.3% (4)
Nalidixic acid	14% (13)
Neomycin	4.3% (4)
Streptomycin	7.5% (7)
Tetracycline	18.3% (17)
Trimethoprim/sulfamethoxazole	0

n, number of resistant isolates.

Table 4. MIC values and PCR analysis of target genes associated with resistance in 13 *Salmonella* isolates displaying (fluoro)quinolone resistance

		MIC values ($\mu g\,mL^{-1}$)		Target gene mutations			
Isolate no./source/serovar	PMQR genes	NAL – NAL+PAβN	CIP	GyrA	GyrB	ParC	ParE
S20/chicken/Infantis	*qnrB19*	32 – 4 (8 ×)	1	WT	WT	Thr57 → Ser	WT
S24/sausages/Uganda	*qnrB19*	32 – 4 (8 ×)	1	WT	WT	Thr57 → Ser	WT
S37/potato/Muenchen	–	1024 – 128 (8 ×)	1	WT	WT	Thr57 → Ser	WT
S38/ground meat/Uganda	*qnrB19*	256 – 64 (4 ×)	1	WT	WT	Thr57 → Ser	WT
S44/ground meat/Anatum	–	1024 – 64 (16 ×)	0.5	Asp87 → Tyr	WT	Thr57 → Ser	WT
S45/meat/Carrau	–	256 – 64 (2 ×)	0.5	Asp87 → Tyr	WT	Thr57 → Ser	WT
S46/cheese/Carrau	–	1024 – 512 (2 ×)	0.5	Asp87 → Tyr	WT	Thr57 → Ser	Asn446 → Pro
S47/ground meat/Uganda	–	1024 – 256 (4 ×)	0.5	WT	WT	Gly25 → Ala Thr57 → Ser	WT
S51/cheese/Carrau	–	1024 – 512 (2 ×)	0.5	Asp87 → Tyr	WT	Thr57 → Ser	WT
S52/ground meat/Carrau	–	1024 – 512 (2 ×)	1	WT	WT	Thr57 → Ser	Arg508 → Lys
S53/ham/Anatum	–	1024 – 512 (2 ×)	0.5	Asp87 → Tyr	WT	Thr57 → Ser	Arg508 → Lys
S64/cheese/Muenchen	–	1024 – 512 (2 ×)	0.5	Asp87 → Tyr	WT	Thr57 → Ser	WT
S75/ground meat/6,7:d:-,	*qnrB19*	256 – 128 (2 ×)	1	WT	WT	WT	WT

NAL, nalidixic acid; CIP, ciprofloxacin; WT, wild type.
Values in parentheses indicate fold MIC reduction in the presence of 40 $\mu g\,mL^{-1}$ PAβN (Phe-Arg-β-napthylamide).

susceptible to ciprofloxacin (MIC = 0.06 μg mL^{-1}), and the latter demonstrated a twofold increased resistance. More recently, Baucheron *et al.* (2005) reported that the Thr → 57Ser ParC substitution was not involved in quinolone resistance in their isolates. Also, Cui *et al.* (2009) reported an identical ParC substitution in a ciprofloxacin-resistant *S.* Rissen isolate that did not carry any other target gene mutation, *qnr* alleles nor an *aac-(6′)-Ib-cr* gene. In addition, the same polymorphism was recently encountered in a number of non-Typhimurium isolates and the resistant phenotype could not be linked with this alteration because susceptible isolates harboured identical mutations (Gunell *et al.*, 2009). Thus, we also sequenced the *parC* gene of 10 randomly selected quinolone-susceptible isolates from this collection representing five serotypes. Thr → 57Ser substitution was identified in nine of 10 of these isolates (data not shown), supporting the view that this is a common polymorphism in serotypes other than Typhimurium. In view of current knowledge regarding quinolone resistance mechanisms, it is unclear whether secondary target mutations alone can lead to the development of high-level quinolone resistance (Ling *et al.*, 2003; Baucheron *et al.*, 2005; Cui *et al.*, 2009; Gunell *et al.*, 2009).

Detection of PMQR

PCR analysis of the fluoroquinolone-resistant isolates did not detect *aac(6′)-Ib-cr*, *qepA*, *qnrA* nor *qnrS* genes. Four isolates were positive for *qnrB* (Table 4): one Infantis (S20), two Uganda isolates (S24, S38) and one serovar 6,7:d:- isolate (S75). The MICs of nalidixic acid in these isolates varied from 32 to 256 μg mL^{-1}. DNA sequencing revealed the presence of the *qnrB19* allele in all cases. Multiple plasmids were present in nine isolates (data not shown) while four other isolates (denoted as S37, S45, S47 and S51) lacked detectable plasmids. In the plasmid-positive *qnrB19* isolates S20, S24, S38 and S75, several other low-molecular-weight plasmids ranging in size between 1 and 3 kb were also noted (data not shown). When analysed by PCR designed to amplify ColE-like plasmids, amplicons of 2.7 kb were recovered. Among these, two distinct MboII RFLP profiles were observed, which were identical for three isolates (S20, S24, and S38), and different for isolate S75 (data not shown). The plasmids from isolates S20 and S75 were purified and completely sequenced.

Homology searches revealed that the plasmid (designated pMK100) found in *S.* Infantis (S20) exhibited 100% homology with *qnrB19*-carrying plasmids including pSGI15, a small ColE plasmid identified recently in *S. enterica* serovar Typhimurium isolated in Germany (Hammerl *et al.*, 2010), and a *qnrB19*-containing plasmid pPAB19 from an *S.* Infantis clinical isolate recovered in Argentina (GenBank accession number GQ412195). The plasmid purified from isolate S75 (designated pMK101) was found to be 97% similar to these latter plasmids. The dissimilarity noted was mapped to an insertion located between nucleotide positions 896 and 957. Remarkably, the latter DNA sequence was identical to one found in a pBC633 from a *K. pneumoniae* strain KN633 (accession number EU176012), a urinary isolate from Colombia displaying carbapenem resistance and reported in 2005. This plasmid of approximately 15.5 kb carried a bla_{KPC-2} gene encoding a class A carbapenemase (Villegas *et al.*, 2006). The additional DNA sequence contained in the plasmid from the isolate S75 was located between the *qnrB19* gene and *orf2*, and was found to be homologous with a region of pBC633. Furthermore, nucleotide sequence similarity was observed in the region upstream of the inserted fragment, possibly facilitating the incorporation of the new DNA fragment. The fact that pBC633 was found only in Colombia indicates that the homology found here may not be coincidental. It is interesting to speculate that pMK101 (the plasmid from isolate S75) is chimeric and may have emerged as a result of a recombination event that led to the horizontal acquisition of a fragment from another plasmid containing bla_{KPC-2}. The process is likely to have occurred in a bacterium simultaneously hosting a plasmid similar to or identical to pBC633, as well as a small ColE-like plasmid such as pMK100. While bla_{KPC-2} genes are frequent in *K. pneumoniae* and only sporadic in other *Enterobacteriaceae*, there are insufficient data to conclude what species was the primary host of the new plasmid structure (Villegas *et al.*, 2006; Pournaras *et al.*, 2009). In addition, it is noteworthy that pBC633 containing a bla_{KPC-2} gene was found on a transposon Tn*4401* with multiple insertion sequence (IS) elements that have likely contributed to its emergence (Naas *et al.*, 2008). Of particular concern is the possibility of the emergence of chimeric plasmids carrying both *qnr* genes and bla_{KPC-2} that could compromise the clinical value of fluoroquinolones and virtually all β-lactams. In view of this, monitoring of phenotypic resistance as well as associated mechanisms and mobility is essential. Furthermore, the occurrence of both bla_{KPC-2} and *qnr* in Colombia and their associated plasmids is likely to be under-reported as a result of poor surveillance as well as diagnostic challenges associated with the low-level resistance conferred (Villegas *et al.*, 2006). The present study documented the emergence of a plasmid carrying a sequence previously identified in another plasmid and suggests that reservoirs of both types of plasmids may exist in Colombia.

In Colombia, epidemiological data relating to PMQR is limited. A single case reporting PMQR in Colombia described the *qnrB19* gene in *E. coli* isolates recovered from blood cultures of a hospital patient in Monteria (Cattoir *et al.*, 2008). The gene was linked with IS*Ecp1*-like insertion element responsible for its mobilization and was carried by a novel transposon designated Tn*2012* identified on pR4525 (Cattoir *et al.*, 2008). No linkage of *qnrB19* with transposon

or integron structures was observed in our isolates (data not shown). A high prevalence of *qnrB* determinants was reported recently in commensal microbial communities cultured from healthy children in Peru and Bolivia (Pallecchi *et al*., 2009). In a follow-up study, the involvement of ColE-type plasmids and their role in dissemination in these two countries was described (Palecchi *et al*., 2010). The most prevalent plasmid, designated pECY6-7, was investigated in detail, and was found to be identical to the plasmid characterized by Hammerl *et al*. (2010). Both plasmids are indistinguishable from those characterized in the *S*. Infantis isolate (denoted as S20).

Concluding remarks

These data extend our understanding of the molecular epidemiology of the *qnrB19* determinant. In this study, the marker was identified for the first time in *Salmonella* spp. in Colombia. The fact that the isolates include different serovars, and that they were recovered in different areas of the country from a variety of food samples and over the years (2002–2009), suggests that the reservoir may not be restricted to a specific ecological niche. Further epidemiological studies are required to determine the full extent of the dissemination of PMQR in Colombia and its implications for public health.

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