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Prevalence of *qnr* genes in *Salmonella* in France

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***Objectives*: To detect the *qnrA*, *qnrB* and *qnrS* genes among *Salmonella* isolates received at the French National Reference Centre for *Salmonella* in Paris, France.**

***Methods*: Antibiotic susceptibility was determined by disc diffusion for 499 *Salmonella* isolates including 320 *Salmonella* Typhimurium, 100 *Salmonella* Enteritidis and 79 *Salmonella* Hadar collected in 2002. Amplification with specific primers of *qnrA*, *qnrB* and *qnrS* genes was performed for all *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Hadar isolates resistant to quinolones and for 17 additional isolates that produced expanded-spectrum β-lactamases (ESBLs).**

***Results*: Prevalence of quinolone resistance was 3.75%, 11% and 79.7% for *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Hadar serovars, respectively. A single isolate (0.2%) was *qnrA*-positive (QnrA1 determinant) being a *Salmonella* serovar Concord carrying also the ESBL gene $bla_{\text{CTX-M-15}}$. This strain was probably from East Africa. No *qnrB* or *qnrS* genes were identified.**

***Conclusions*: Whereas plasmid-mediated quinolone resistance of the Qnr type is emerging in Enterobacteriaceae worldwide, it remains rare in *Salmonella* in France.**

Keywords: serovar Concord, *qnrA1*, CTX-M-15

Introduction

Salmonella constitutes an important cause of human food-borne gastroenteritis, which may be treated with fluoroquinolones in the case of immunocompromised patients or systemic infections. Although resistance to fluoroquinolones remains rare in *Salmonella*, reduced susceptibility is increasing worldwide.[1,2] It has been suggested that this may be the result of selection of animal isolates and their transfer to humans via the food chain.[3] A European survey performed in 2000 showed that resistance to ciprofloxacin remains rare in *Salmonella* (0.5%) but resistance to nalidixic acid associated with decreased susceptibility to ciprofloxacin (MICs from 0.25 to 1.0 mg/L) was found in 14% of isolates ($n = 27\,059$).[4]

Acquired resistance to quinolones mainly results from chromosomal mutations responsible from modifications of DNA gyrase and topoisomerase IV and from a decrease in drug accumulation in bacteria mainly as a result of the overexpression of efflux pumps (e.g. AcrAB-TolC system).[2] Recently, plasmid-mediated resistance of Qnr type (QnrA, B and S) has been identified in several enterobacterial species including *Salmonella* spp.[5–8] Qnr are pentapeptide repeat proteins that may prevent quinolones from binding to DNA topoisomerases. These proteins confer resistance to nalidixic acid and reduced susceptibility to fluoroquinolones.[8]

The aim of this study was to determine the spread of *qnrA*, *qnrB* and *qnrS* genes in human *Salmonella* isolates obtained from the French National Reference Centre for *Salmonella* (NRC-Salm)

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collected in 2002. We report here a *Salmonella enterica* serovar Concord isolate harbouring the QnrA determinant.

Materials and methods

Bacterial isolates

In 2002, a total of 11 775 human *Salmonella* isolates were registered at the NRC-Salm and 74.9% of them belonged to the three most frequently encountered serovars in France: Enteritidis (37.9%, $n = 4469$), Typhimurium (33.9%, $n = 3998$) and Hadar (2.4%, $n = 282$).[9] Out of those isolates, 499 representative strains (320 *Salmonella* Typhimurium, 100 *Salmonella* Enteritidis and 79 *Salmonella* Hadar) were available in the NRC-Salm and were studied. Moreover, because of frequent association of genes coding for expanded-spectrum β-lactamases (ESBLs) and *qnr* genes, all *Salmonella* isolates resistant to extended-spectrum cephalosporins ($n = 17$) were also tested. These 17 additional isolates collected from 2001 to 2005 belonged to the following serovars: Typhimurium ($n = 3$), Virchow ($n = 3$), Newport ($n = 2$), Concord ($n = 2$), Paratyphi B ($n = 1$), Livingstone ($n = 1$), Bareilly ($n = 1$), Enteritidis ($n = 1$), Agona ($n = 1$), Panama ($n = 1$) and Westhampton ($n = 1$). *Escherichia coli* ATCC 25922 and azide-resistant *E. coli* J53 were used as the control for susceptibility testing and as the host in conjugation experiments, respectively. *E. coli* Lo, *Klebsiella pneumoniae* Kp25 and *E. coli* (pBCH2.6) were used as *qnrA*-, *qnrB*- and *qnrS*-positive controls, respectively.

Susceptibility testing

Antibiotic susceptibility was determined by disc diffusion on Mueller–Hinton agar according to CLSI guidelines.[10] The discs were supplied by Bio-Rad Laboratories (Marnes-la-Coquette, France) and the following antibiotics were tested: amoxicillin (25 μg), ticarcillin (75 μg), amoxicillin/clavulanate (20/10 μg), ticarcillin/clavulanate (75/10 μg), piperacillin (75 μg), piperacillin/tazobactam (75/10 μg), cefalotin (30 μg), cefuroxime (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), aztreonam (30 μg), latamoxef (30 μg), cefepime (30 μg), imipenem (10 μg), kanamycin (30 IU), tobramycin (10 μg), gentamicin (15 μg), netilmicin (30 μg), amikacin (30 μg), nalidixic acid (10 μg), norfloxacin (5 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), tetracycline 30 (IU), fosfomycin (50 μg), rifampicin (30 μg), sulfamethoxazole (200 μg), trimethoprim (5 μg) and colistin (50 μg). MICs of quinolones, fluoroquinolones and β-lactams were determined by using the Etest method according to the manufacturer's recommendations (AB Biodisk, Solna, Sweden). MIC breakpoints used for susceptibility and resistance to nalidixic acid and ciprofloxacin were $\leq$8 and $\geq$32 mg/L and $\leq$1 and $\geq$4 mg/L, respectively, as recommended by the CLSI.[10]

PCR amplification and sequencing

Amplification of the *qnrA*, *qnrB* and *qnrS* genes was performed for all *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Hadar isolates categorized as intermediate or resistant to nalidixic acid and/or ciprofloxacin and for the 17 ESBL-positive isolates. Genomic DNA was extracted using the InstaGene matrix kit (Bio-Rad, Marnes-la-Coquette, France) in accordance with the manufacturer's recommendations. PCR experiments were carried out according to standard conditions (annealing temperature at 55°C, extension 1 min at 72°C, 35 cycles) using primers synthesized by Proligo France SAS. Primers used were as follows: for *qnrA*, QnrA-A (5′-GGGTAT GGATATTATTGATAAAG-3′) and QnrA-B (5′-CTAATCCG GCAGCACTATTA-3′) to give a 660 bp product; for *qnrB*, QnrBm-F (5′-GGMATHGAAATTCGCCACTG-3′) and QnrBm-R (5′-TTTGCYGYYCGCCAGTCGAA-3′) (able to amplify the *qnrB1* to *qnrB6* variants) to give a 264 bp product; and for *qnrS*, QnrS-A2 (5′-AGTGATCTCACCTTCACCGC-3′) and QnrS-B2 (5′-CAGGCTGCAATTTTGATACC-3′) to give a 550 bp product. The quinolone-resistance determining regions (QRDRs) of *gyrA*, *gyrB*, *parC* and *parE* genes of the *qnrA*-positive isolate were amplified using previously described primers.[11] Detection and identification of ESBL was performed for the *qnrA*-positive strain (and its transconjugant) using specific primers of sequences encoding TEM-, SHV- and CTX-M-type ESBLs, as previously described.[9,12] After PCR amplification, DNA fragments were purified with the Qiaquick PCR purification kit (Qiagen, Courtaboeuf, France). Both strands of the amplification products obtained were sequenced with an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analysed with software available over the Internet at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Conjugation, plasmid analysis and PCR mapping

Conjugation experiments using an azide-resistant *E. coli* J53 (Azi^R) as recipient were performed in liquid and solid culture media. Transconjugants were selected on Trypticase soy (TS) agar plates containing sodium azide (100 mg/L) plus amoxicillin (50 mg/L) or nalidixic acid (16 mg/L). Plasmid analysis of the *qnrA*-positive strain and its *E. coli* transconjugants was performed by using the Kieser technique followed by agarose gel electrophoresis analysis.[13] The sequence of the DNA adjacent to the *qnrA* gene was determined by PCR mapping using different primers sets. Primers specific for the *qnrA* gene (see above) were used in combination with primers 3′CS (5′-AAGCA GACTTGACCTGA-3′), 5′CS (5′-GGCATCCAAGCAG CA AG-3′), ORF513-D3 (5′-CTCACGCCCTGGCAAGGTTT-3′), ORF513-D5 (5′-CTTTTGCCCTAGCTGCGGT-3′) and ampR- 5′ext (5′- GCGGGTAAAACTGAGATGAC-3′).

Results and discussion

Prevalence of quinolone resistance

Twelve out of the 320 isolates of *Salmonella* Typhimurium isolates (3.75%) were resistant to nalidixic acid (with MICs of ciprofloxacin ranging from 0.25 to 0.5 mg/L) and only a single isolate (0.3%) was resistant to fluoroquinolones (MIC $>$ 32 mg/L). Out of the 100 isolates of *Salmonella* Enteritidis and 79 of *Salmonella* Hadar, 11 (11%) and 63 (79.7%) isolates were resistant to nalidixic acid, respectively. Among the 17 isolates resistant to extended-spectrum cephalosporins, only three were resistant to nalidixic acid and none to ciprofloxacin.

As reported in the literature, we found that resistance to nalidixic acid is mostly in *Salmonella* Hadar (57%) and is less frequently observed in the two most frequently isolated serovars Enteritidis and Typhimurium (13% and 8%, respectively).[4] We also reported herein that high-level resistance to fluoroquinolones remains very uncommon in *Salmonella*, generally lower than 1% of isolates.[2,4]

Screening for the qnr genes

For all quinolone-resistant isolates of *Salmonella* Typhimurium ($n = 13$), *Salmonella* Enteritidis ($n = 11$) and *Salmonella* Hadar

Table 1. MICs of 10 antibiotics for the *Salmonella enterica* serovar Concord clinical isolate 05-3728, its transconjugant and reference strain *E. coli* J53 Az^R

Antibiotic	MIC (mg/L) for strain		
	Salmonella Concord	*E. coli*	
	05-3728	transconjugant (*qnrA1*+, $bla_{CTX\text{-}M\text{-}15}$+)	J53 Az^R
Nalidixic acid	32	32	4
Norfloxacin	2	2	0.06
Ofloxacin	4	2	0.12
Ciprofloxacin	0.5	0.5	0.12
Moxifloxacin	2	2	0.06
Sparfloxacin	2	2	0.03
Aztreonam	>256	>256	0.12
Cefotaxime	>32	>32	4
Ceftazidime	>256	>256	0.06
Ceftazidime/clavulanic acid	2	0.5	<0.06

($n = 63$), screening gave negative results for *qnrA*, *qnrB* and *qnrS* genes. Among the 17 strains resistant to extended-spectrum cephalosporins, one isolate had a *qnrA*-like gene but none of these was positive for the *qnrB* and *qnrS* genes. This isolate (05-3728) was obtained from two children from the same family and belonged to the serovar Concord. Isolate 05-3728 was recovered in June 2005 from stool of a 2.5-year-old child hospitalized for severe acute gastroenteritis. This episode appeared 2 months after the arrival in his family of an adopted 7-month-old girl from Ethiopia. Even though she was asymptomatic, a rectal swab screening was performed that led to isolation of an isolate presenting an antibiotic resistance pattern identical to that of the isolate 05-3728. Molecular typing by PFGE gave similar patterns for these two isolates and confirmed clonal relatedness between them (data not shown). Interestingly, no quinolone-based treatment had been previously given to these children.

Out of the 516 tested isolates, only a single isolate was positive for *qnrA* (0.2%). An earlier study, performed in South Korea, did not identify the *qnrA* gene among 261 nalidixic acid-resistant and community-acquired *Salmonella* spp. isolates.[1] In the second study, 10 out of 335 *Salmonella* human clinical isolates from the USA were positive for either *qnrB* or *qnrS* but none was *qnrA*-positive.[5] An that latter study, several *qnrB* variants were detected in seven *Salmonella* Berta isolates (*qnrB5*) and one *Salmonella* Mbandaka isolate (*qnrB2*), whereas *qnrS* variants were found in isolates of *Salmonella* Anatum (*qnrS2*) and *Salmonella* Bovismorbificans (*qnrS1*).[5] All of these 10 isolates were resistant to nalidixic acid with decreased susceptibility to ciprofloxacin. In addition, QnrA-like determinants were detected in four unrelated clinical isolates of *Salmonella* Enteritidis in Hong Kong that harboured a $bla_{CTX\text{-}M\text{-}14}$ gene and QnrS1 determinant was recovered in a *Salmonella* Infantis of avian origin in Germany.[6,7]

Characterization of the qnrA-positive Salmonella Concord isolate

Isolate 05-3728 was resistant to all β-lactams, except carbapenems and cephamycins, and exhibited an ESBL phenotype. PCR and sequencing revealed that it harboured the *qnrA1* and the $bla_{CTX\text{-}M\text{-}15}$ genes. Thus, like most *qnrA*-positive enterobacterial isolates reported worldwide, this *qnrA*-positive isolate expressed an ESBL.[8,12] It was resistant to several aminoglycosides (kanamycin, gentamicin and tobramycin) but remained susceptible to amikacin and netilmicin. In addition, it was also resistant to chloramphenicol, tetracycline, rifampicin, sulphonamide and trimethoprim, and remained susceptible to fosfomycin. Finally, it was resistant to nalidixic acid (MIC >32 mg/L) but remained susceptible to ciprofloxacin (MIC 0.38 mg/L) (Table 1).

Interestingly, no mutation linked to quinolone resistance was detected in the QRDRs of *gyrA*, *gyrB*, *parC* and *parE* genes of this isolate. Quinolone resistance was transferred to *E. coli* J53 Azi^R by conjugation. The transconjugant exhibited a similar pattern of resistance for almost all antimicrobial agents, in particular to quinolones (Table 1), but was susceptible to aminoglycosides. PCR and sequencing confirmed that the transconjugant carried $bla_{CTX\text{-}M\text{-}15}$ and *qnrA1* genes. Plasmid analysis revealed that isolate 05-3728 contained five plasmids with estimated sizes of approx. 200, 100, 50, 15 and 5 kb whereas the transconjugant contained a single *qnrA1*(+) 200 kb plasmid (data not shown). The *qnrA1* gene was located on a *sul1*-type class 1 integron that was identical to the In36 integron identified in *E. coli* isolates from Shanghai.[14] Briefly, the *orf513* gene was found immediately upstream of the *qnrA1* gene, and an *ampR* sequence coding for a LysR-type regulatory element was identified downstream.

We report here the prevalence of plasmid-mediated quinolone resistance in *Salmonella* in France. Only a single isolate (0.2%) was positive for the *qnrA* gene and none was positive for *qnrB* and *qnrS* genes. These results may indicate a low-level rate of dissemination of Qnr determinants among human *Salmonella* isolates.

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Transparency declarations

None to declare.

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