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Human isolates of *Salmonella enterica* serovars Hadar, Kentucky, Virchow, Schwarzengrund, and the monophasic variant of *S. Typhimurium*, *Salmonella enterica* subsp. *enterica* serovar 4,5,12:i− were examined for mutations within the quinolone resistance target genes *gyrA*, *gyrB*, *parC*, and *parE* and for plasmid-mediated resistance genes. Differences were observed among the serovars. A novel variant of *qnrD, qnrD2* was detected in an S. Hadar isolate.

The main mechanism of quinolone resistance is the accumulation of mutations in DNA gyrase and DNA topoisomerase IV genes, with plasmid-mediated quinolone resistance (PMQR) additionally conferring a form of reduced susceptibility (1). PMQR genes include *qnrA, qnrB, qnrC, qnrD, qnrS* (1), and *qnrVC* (2), which encode the Qnr families of pentapeptide repeat proteins, the modified aminoglycoside acetyltransferase gene *aac(6′)-Ib-cr*, as well as *qepA* and *oqxAB* (1), which encode the efflux pumps QepA and OqxAB, respectively. High-level fluoroquinolone resistance among *Salmonella* isolates from humans has been reported in Europe (3–6), although at relatively low rates (7).

This study provides a survey of quinolone resistance determinants in human isolates of *Salmonella enterica* serovars Hadar, Kentucky, Virchow, Schwarzengrund, and the monophasic variant of *S. Typhimurium*, *S. enterica* subsp. *enterica* serovar 4,5,12:i− in Switzerland, and reports a novel PMQR gene, *qnrD2*. (This work was presented in part at the 4th ASM Conference on *Salmonella*, Boston, MA, 2013.)

Forty-eight nalidixic acid-resistant *Salmonella* species isolates with different resistance profiles to other antibiotics collected between 2005 and 2011 from diseased humans were obtained from the National Centre for Enteropathogenic Bacteria and Listeria, Zurich, Switzerland. Ten S. Hadar isolates (8), 17 S. Kentucky isolates (9), 13 S. Virchow isolates (10), 6 S. enterica serovar 4,5,12:i− isolates (11), and 2 S. Schwarzengrund isolates were selected. Three nalidixic acid-susceptible S. Hadar isolates, 3 S. Kentucky isolates, 3 S. Virchow isolates, 3 S. enterica serovar 4,5,12:i− isolates, and two S. Schwarzengrund isolates from the same collection served as control strains. In most cases, the isolates were clonally unrelated or belonged to a large cluster (Table 1). Five of the S. Hadar isolates belonged to a subcluster (Table 1) with indistinguishable pulsed-field gel electrophoresis (PFGE) patterns (8).

Amplification and sequencing of the target genes using primers described previously were performed for *gyrA*, *gyrB*, *parC*, and *parE* (12) and for the plasmid-mediated quinolone resistance genes *qnrA* (12, 13), *qnrB* (14), *qnrC* (15), *qnrD* (16), *qnrS*, *aac(6′)-Ib-cr*, and efflux pump gene *qepA* (12). For sequencing *qnrB* and *qnrS*, the newly designed primers qnrB_seq_F (seq stands for sequencing, and F stands for forward) (CAC TTA TGC ATG GGA CGG), qnrB_seq_R (R stands for reverse) (CGG ATT TGA CGC ATA ACC), qnrS1_orf_r (TGT GTA ATG TGT GTAT GTA ACA GG), and qnrS1_orf_r (CCC TAT GTC TAT TGC AAC AAG) were used. Conjugational transfer was performed by a standard broth mating method, using *Escherichia coli* strain HK225 as the recipient (17). Cloning using the vector plasmid pUC19 and electrocompetent *E. coli* ATCC 25922 cells and sequencing were performed using the conditions listed in Table S1 in the supplemental material. Susceptibility was determined using Etest strips (bioMérieux, Marcy l’Etoile, France) and the disk diffusion and microdilution methods (18). For selected isolates, microdilution was performed in the absence and presence of the efflux pump inhibitor phenylalanine–arginine–β-naphthylamide (PAβN) at a final concentration of 40 μg/ml.

The results of this study are summarized in Table 1. Target gene mutations were not distributed equally among the serovars. Among S. Hadar isolates, alterations in GyrA appeared most commonly (90%) as single-amino-acid substitutions. Only isolates containing the substitution Ser80→Ile in ParC, i.e., 15 S. Kentucky isolates and one S. Hadar isolate (N10-1465) were resistant to ciprofloxacin according to CLSI criteria (18). As no mutations in *parC* were detected in S. Virchow, S. Schwarzengrund, or *S. enterica* serovar 4,5,12:i−, this finding indicates that topoisomerase IV mutations are possibly characteristic of S. Kentucky and may occur more frequently than previously understood (19), although it may be due to the fact that most of the isolates belong to the same large cluster (9).

All the ciprofloxacin-resistant S. Kentucky isolates contained the substitution Ser83→Phe in GyrA, and six isolates
Isolates belonging to a subcluster with indistinguishable PFGE profiles (8).

The MICs of nalidixic acid (NAL) alone and NAL with phenylalanine–arginine–
cetylosphingosine–naphthylamide (PA-CNS-NAP) were determined by microdilution.

### Table 1: Quinolone Resistance in Salmonella

<table>
<thead>
<tr>
<th>Year</th>
<th>Hospital</th>
<th>NAL MIC (µg/ml)</th>
<th>CIP MIC (µg/ml)</th>
<th>CFE MIC (µg/ml)</th>
<th>CTX MIC (µg/ml)</th>
<th>CFX MIC (µg/ml)</th>
<th>POX MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Hadar</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td>0.5</td>
<td>0.064</td>
</tr>
<tr>
<td>2006</td>
<td>Hadar</td>
<td>0.19</td>
<td>0.094</td>
<td>0.064</td>
<td>0.094</td>
<td>0.19</td>
<td>0.064</td>
</tr>
<tr>
<td>2007</td>
<td>Hadar</td>
<td>0.125</td>
<td>0.094</td>
<td>0.064</td>
<td>0.094</td>
<td>0.125</td>
<td>0.064</td>
</tr>
<tr>
<td>2008</td>
<td>Kentucky</td>
<td>1.024</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>1.024</td>
<td>0.25</td>
</tr>
<tr>
<td>2009</td>
<td>Hadar</td>
<td>0.094</td>
<td>0.064</td>
<td>0.025</td>
<td>0.064</td>
<td>0.094</td>
<td>0.025</td>
</tr>
<tr>
<td>2010</td>
<td>Hadar</td>
<td>0.125</td>
<td>0.094</td>
<td>0.064</td>
<td>0.094</td>
<td>0.125</td>
<td>0.064</td>
</tr>
<tr>
<td>2011</td>
<td>Kansas</td>
<td>1.024</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>1.024</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Notes:**
- NAL: Nalidixic Acid
- CIP: Ciprofloxacin
- CFE: Ceftazidime
- CTX: Cefotaxime
- CFX: Cefixime
- POX: Cepapodoxime
- CEF: Cefepime
- PNX: Pemexilime
- SXT: Trimethoprim-Sulfamethoxazole

**Coresistance:**
- qnrS1
- qnrB2
- qnrD2

**Resistance profiles, target gene mutations, prevalence of PMQR, and effect of efflux pump inhibitor for isolates described in this study.**
(N05-1087, N06-0062, N06-1493, N06-1616, N07-0953, and N08-1057) contained mutations in codon Asp87, which has been reported to be useful for tracking the geographical origin of the pandemic ciprofloxacin-resistant S. Kentucky strain 10198 (20).

All S. Virchow isolates uniformly revealed an Asp87→Tyr substitution in GyrA. As all but two (N05-2379 and N07-2461) of the isolates belong to a large cluster found to be disseminated internationally (10), this suggests that S. Virchow maintains a highly established quinolone target gene resistance genotype. Interestingly, this serovar contained three of the four detected plasmid-mediated resistance genes (two qnrS1 genes and one qnrB2 gene). Isolates N06-978 and N07-0090 harboring qnrS2 plasmids belong, together with a qnrS2-harboring S. Virchow isolate described previously (6), to a subcluster associated with travel to Thailand (10).

One S. Hadar isolate (N08-2312) harbored the plasmid-mediated quinolone resistance gene qnrD2, a novel variant of qnrD1 (16). Although differing from qnrD1 by two amino acid exchanges (Ile189→Thr and Leu202→Phe), no effect on resistance levels to quinolones was detected when comparing isogenic E. coli strains containing cloned qnrD1 and qnrD2, respectively. The MICs of nalidixic acid and ciprofloxacin were 1 μg/ml and 0.03 μg/ml, respectively, in both cases (see Table S2 in the supplemental material). Sequencing of the complete plasmid containing qnrD2 revealed a 4,268-bp plasmid (pQnrD2) with 99% homology to p2007057 containing qnrD1 (16). Apart from the qnrD gene, this plasmid contains 4 open reading frames of unknown function. None of the control strains contained target gene mutations or PMQR genes.

Conjugational transfer was successful for S. Virchow N06-0978 containing qnrS1, but not for the other isolates containing qnrD2, qnrS1, and qnrB2. As expected (21), the efflux pump inhibitor PAβN was not sufficient to restore antibiotic susceptibility, although it caused a 32-fold reduction of nalidixic acid MICs in 12.5% of the tested isolates (Table 1).

This study documents quinolone resistance mutations in different Salmonella serovars, which may be useful for understanding the origins and routes of dissemination of resistant isolates.

Nucleotide sequence accession numbers. The nucleotide sequences of qnrD2 and pQnrD2 have been deposited at GenBank under accession numbers KF055448 and KJ158441, respectively.

ACKNOWLEDGMENTS

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