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Letter to the Editor

Multiple Resistance Mechanisms in Fluoroquinolone-Resistant *Salmonella* Isolates from Germany

During the early 1990s, a clone of multiresistant *Salmonella enterica* serovar Typhimurium O5 negative (var. Copenhagen) of phage type DT204c predominated in Germany and neighboring countries within the calf-fattening industry. In addition to being resistant to ampicillin, kanamycin, tetracycline, chloramphenicol, and trimethoprim, these isolates were highly resistant to nalidixic acid (MIC > 128 μg/ml) and ciprofloxacin (MIC = 32 μg/ml) (4). Heisig et al. (3) showed that the clone had spread among humans and animals in Germany, and complementation experiments suggested that mutations in *gyrA* and *gyrB* were responsible for the high level of fluoroquinolone resistance. DNA sequencing of *gyrA* revealed two amino acid substitutions (Ser83→Ala and Asp87→Asn) (3).

Our recent molecular characterization of fluoroquinolone-resistance mechanisms in contemporary and old *Salmonella* strains showed that two such German isolates of bovine origin carried these *gyrA* mutations and a novel *gyrB* mutation together with a novel *parC* mutation in each strain. Both isolates (NRL608/93 and NRL154/94) were received at the German National Salmonella Reference Laboratory in the early 1990s, and the following MICs of fluoroquinolones were observed by the standard NCCLS broth dilution method (7): ciprofloxacin, 32 μg/ml; enrofloxacin, >128 μg/ml; levofloxacin, 16 and 8 μg/ml; moxifloxacin, 32 μg/ml; and ofloxacin, >128 μg/ml. In order to investigate the underlying resistance mechanisms, the quinolone resistance-determining regions (QRDRs) were amplified by using specific primers for *gyrA* and *parC* (6) and *gyrB* (F, ACTGGCGGACTGTCAGGAAC; B, TCTGACGATA GAAGAAGGTCAAC). Sequencing was carried out as described in reference 6. The most relevant findings were as follows. (i) Sequence analysis of the QRDR of the *gyrA* gene detected the mutations Ser83→Ala (TCC→GCC) and Asp87→Asn (GAC→AAC). (ii) Sequence analysis of the QRDR of *gyrB* showed in both isolates the novel mutation Ser464→Phe (TCC→TTC). This change may alter the hydrophobicity of the protein. In *Salmonella*, the substitution Ser464→Tyr in *gyrB*, which does not affect the local charge or hydrophobicity of the protein, had been described most frequently (2, 8). (iii) Sequence analysis of the QRDR of *parC* showed in both isolates the mutation Ser80→Ile (AGC→ATC). This *parC* substitution has frequently been described for high-level resistance to fluoroquinolones in *Escherichia coli* in combination with *gyrA* double mutations (8). Recently, Baucheron et al. (1) described this mutation for Belgian *Salmonella* isolates. (iv) Phenotypic analysis of the tolerance to the organic solvent cyclohexane (5) showed that both isolates were resistant. This cyclohexane resistance suggests the possession of broad-spectrum efflux pumps implicated in the high resistance to the hydrophilic fluoroquinolones as well. Although the contributions of the various genetic mechanisms described above to the fluoroquinolone resistance phenotype were not elucidated in our study, genetic work in other species (8) suggests their relevance.

The description of this particular clone is of special importance, since it was part of a wide animal outbreak and sporadic human infections (4). The isolation of multiresistant strains from cattle with at least four different genetic backgrounds (double mutation in *gyrA*, single mutation in *gyrB*, single mutation in *parC*, and the presumable presence of efflux pumps) implicated in fluoroquinolone resistance should be a cause of concern and be prevented in the future by decreasing selective pressure.

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REFERENCES

1. **Baucheron, S., H. Imberechts, E. Chaslus-Dancla, and A. Cloeckaert.** 2002. The AcrB multidrug transporter plays a major role in high level fluoroquinolone resistance in *Salmonella enterica* serovar Typhimurium phage type DT204. Microb. Drug. Res. **8:**281–289.
2. **Cloeckaert, A., and E. Chaslus-Dancla.** 2001. Mechanisms of quinolone resistance in *Salmonella*. Vet. Res. **32:**291–300.
3. **Heisig, P., B. Kratz, E. Halle, Y. Graser, M. Altwegg, W. Rabsch, and J. Faber.** 1995. Identification of DNA gyrase A mutations in ciprofloxacin-resistant isolates of *Salmonella typhimurium* from men and cattle in Germany. Microb. Drug. Res. **1:**211–218.
4. **Helmuth, R.** 2000. Antibiotic resistance in *Salmonella*, p. 89–106. *In* C. Wray and A. Wray (ed.), *Salmonella* in domestic animals. CABI Publishing, Oxon, United Kingdom.
5. **Liebana, E., C. Clouting, C. A. Cassar, L. P. Randall, R. A. Walker, E. John Threlfall, F. A. Clifton-Hadley, A. M. Ridley, and R. H. Davies.** 2002. Comparison of *gyrA* mutations, cyclohexane resistance, and the presence of class I integrons in *Salmonella enterica* from farm animals in England and Wales. J. Clin. Microbiol. **40:**1481–1486.
6. **Malorny, B., A. Schroeter, B. Guerra, and R. Helmuth.** Incidence of quinolone resistance in veterinary *Salmonella* strains isolated in Germany between 1998 and 2001. Vet. Rec., in press.
7. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **Piddock, L. J. V.** 2002. Fluoroquinolone resistance in *Salmonella* serovars isolated from human and food animals. FEMS Microbiol. Rev. **26:**3–16.

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