Quinolone Resistance-Determining Regions of gyrA and parC in *Pasteurella multocida* Strains with Different Levels of Nalidixic Acid Resistance

*Pasteurella multocida* causes sporadic or epidemic diseases among different animal species, including feral and systemic infections in humans. Penicillin is the drug of choice for treatment of *Pasteurella* infections, but third-generation cephalosporins and fluoroquinolones are a good alternative for beta-lactamase-producing strains or for allergic patients (1, 10).

Quinolone resistance in gram-negative bacteria is increasing, with different mutations occurring in the quinolone resistance-determining regions (QRDR) of the *gyrA* and *parC* genes, one of the main causes of resistance (3, 5–9). In an attempt to determine if this mechanism also occurs in *P. multocida*, both QRDR were isolated and sequenced from six isolates from animal (PM25 and its derivative PM1024) and human clinical (16Q, 14Q, and 15Q) origins (Table 1). All strains were identified by standard methods (4), MICs were determined by the E-test method (AB Biodisk), and the epidemiological relationship of strains was corroborated by pulsed-field gel electrophoresis (data not shown). Strains PM25 and 16Q were fully susceptible to all quinolones assayed, while PM1024, 14Q, and 15Q presented different levels of nalidixic acid resistance (Table 1).

PCR amplification with degenerate oligonucleotide primers, described below for *Haemophilus influenzae*, was used to amplify the QRDR of the *gyrA* and *parC* genes of strain PM25, which were further sequenced (3). The nucleotide sequences of both QRDR (accession numbers AF173979 and AF173980 of GenBank for *gyrA* and *parC*, respectively) were compared with data from The Institute for Genomic Research (http://www.tigr.org). Identities found were 98 and 96% with *P. multocida* PM70, 83 and 82% with *Actinobacillus actinomycetemcomitans*, 81 and 85% with *H. influenzae*, and 78 and 78% with *Escherichia coli* for the QRDR of the *gyrA* and *parC* genes, respectively. PM25 nucleotide sequences obtained were used to design specific primers to amplify both QRDR of the other strains (*gyrA*, 5′-GATGACGAAAGGCGGGAATGCGC-3′ and 5′-CCGTAATGCCTCCGGTATG-3′, and *parC*, 5′-GAAGCTTGGTTTAAATGCCGCC-3′ and 5′-CTCGACTGCCATATTTT-3′, at amplicon positions in *E. coli* of 153 to 535 and 151 to 493, respectively). A comparison of the deduced amino acid sequences of both QRDR with *E. coli* revealed two changes in the GyrA subunit: a Ser-to-Ile mutation (AGC→ATC) in PM1024 and an Asp-to-Gly mutation (GAC→GGC) in 14Q and 15Q at positions exactly analogous to Ser-83 and Asp-87 of *E. coli*, respectively (Table 1). These mutations may be responsible for the different levels of nalidixic acid resistance and for the decreased susceptibilities to fluoroquinolones that these strains exhibit. However, other mechanisms could be involved in the MIC increase, because after four subcultures of strains 14Q, 15Q, and PM1024 in 5% blood agar (Oxoid) without quinolones, nalidixic acid MICs were 2.0, 2.0, and 48 μg/ml, respectively. Therefore, mutations found could be the starting point for additional changes that, in conjunction with other mechanisms, could lead to a high level of fluoroquinolone resistance in *P. multocida*.

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REFERENCES


