Fluoroquinolones are broad-spectrum antibacterial agents that inhibit DNA gyrase and topoisomerase IV activities (9, 11). Both enzymes are composed of two A and two B subunits, encoded by the gyrA and gyrB genes for DNA gyrase and parC and parE genes for topoisomerase IV, respectively. We reported previously (1, 3) that three of these genes, gyrA, parC, and parE, were associated with fluoroquinolone resistance in Mycoplasma hominis. However, these studies focused only on mutants selected in vitro. We have now characterized five clinical isolates of M. hominis which exhibited high-level resistance to various fluoroquinolones.

The first isolate, MHa, was isolated from the genitourinary tract of a 35-year-old AIDS patient treated successively with ofloxacin, ciprofloxacin, and sparfloxacin for infections associated with a chronic obstructive airway disease. The second and third isolates (MHb1 and MHb2) were isolated on two distinct occasions (6 months apart) from lung aspirates from the same patient. The fourth and fifth isolates, MHc1 and MHc2, respectively, were isolated, at the same time, from a bronchoalveolar lavage specimen and laparotomy pus of a 25-year-old immunocompromised woman presenting for respiratory distress syndrome. She received three sequential treatments, 1 month each, with ciprofloxacin for occasional fevers complicating a bone marrow transplantation.

Growth conditions and antibiotic susceptibility testing of the M. hominis strains have been previously described (1). Chromosomal DNA of each of the five strains was used as a template in a PCR to amplify the quinolone resistance-determining regions (QRDRs) of the gyrA, gyrB, parC, and parE genes as previously described (1, 3). The 5’ ends of the gyrB and parE genes were amplified with primers GBF (5’-CTTGGCTATGA GAATAAGTGAATTT) and GBR (5’-ACGTGTTTACAAA ACTTTG-3’) for gyrB (12) and PEF (5’-CATCCTTATTA GTGGAAAGT-3’) and PER (5’-ATTCAATAGTGAATTGGC GCGA-3’) for parE (2).

Table 1 summarizes the analyses of the five clinical isolates.
of *M. hominis* in comparison to the *M. hominis* reference strain, PG21. All five isolates had a similar high level of quinolone resistance (8- to 16-fold-increased MICs of ofloxacin and ciprofloxacin and a 50- to 100-fold-increased MIC of sparflloxacin) and were found to carry mutations in both GyrA and ParC or ParE subunits.

In GyrA, isolate MHa harbored a Glu87-to-Lys change (*Escherichia coli* numbering), whereas isolates MHb1, MHb2, MHc1, and MHc2 had Ser83-to-Leu substitutions. In addition, nucleotide sequence comparisons revealed that the five clinical isolates harbored *gyrA* silent mutations, compared with the reference strain, PG21, at positions corresponding to residues 66, 80, 101, 106, 128, 132, and 150. In *M. hominis*, the Ser83-to-Leu change in GyrA was also found in fluoroquinolone-resistant mutants selected in vitro. In contrast, the Glu87-to-Lys substitution selected in the clinical isolate MHa was found in none of the in vitro-selected mutants (1, 3).

In ParC, two amino acid alterations were found at positions 81 and 84 (*E. coli* numbering). The Glu84-to-Gly substitution was found in MHa, whereas the Ser81-to-Pro change occurred in strains MHb1 and MHb2. Silent mutations were also found at positions 84 and 85. In laboratory mutants of *M. hominis* resistant to fluoroquinolones, residue 84 was found to be the site of the most prevalent substitution (3). The ParC Ser-to-Pro change found in isolates MHb1 and MHb2 at position 81, a position much less frequently altered than positions 80 and 84, has also been described to occur in clinical isolates of *Neisseria gonorrhoeae* (7).

Determination of the novobiocin susceptibility of the five fluoroquinolone-resistant mutants (Table 1), novobiocin MICs for three isolates, MHa, MHc1, and MHc2, showed a fourfold increase. For these strains, the 5’ region of *gyrB*, including the novobiocin resistance loci described for other species (5, 13), and the homologous 5’ region of *parE* were amplified and sequenced without revelation of any base change compared with the reference strain PG21. A possible active efflux system affecting the novobiocin MIC could be implicated.

Interestingly, isolates MHb1 and MHb2, isolated 6 months apart from the same patient, exhibited identical profiles of resistance to fluoroquinolones, novobiocin, and josamycin, a

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**TABLE 2. Amino acid changes in ParE (GyrB) QRDR associated with quinolone resistance**

<table>
<thead>
<tr>
<th>Species</th>
<th>Type</th>
<th>ParE (GyrB) sequence or mutation position</th>
</tr>
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<tbody>
<tr>
<td><em>M. hominis</em></td>
<td>Mutant</td>
<td>ACVFEKVIQEGDSAGGSAKLGRNRVTQAILPLRGKVI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INTDKAKLSDVLANEIEATIINTIGAGIGEDFNL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66-132</td>
</tr>
<tr>
<td></td>
<td>PG21</td>
<td>ACVFEKVIQEGDSAGGSAKLGRNRVTQAILPLRGKVI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INTDKAKLSDVLANEIEATIINTIGAGIGEDFNL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66-132</td>
</tr>
<tr>
<td></td>
<td>*D426</td>
<td>ACVFEKVIQEGDSAGGSAKLGRNRVTQAILPLRGKVI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INTDKAKLSDVLANEIEATIINTIGAGIGEDFNL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66-132</td>
</tr>
</tbody>
</table>

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As with the in vitro mutants of *M. hominis* (1, 3), none of the five clinical isolates was found to carry an alteration in the GyrB QRDR, although silent mutations were identified at positions 427, 435, 457, and 460. This clearly confirms the prevalence of mutations in *gyrA* over those in *gyrB*. However, it should be noticed that the amino acid residue at position 426 in the GyrB subunit of the reference strain, *M. hominis* PG21, is already an asparagine (12), that amino acid always substituting for the aspartic acid at position 426 in the GyrB QRDR of quinolone-resistant bacteria previously described.

Determining the status of the ParE QRDRs revealed that isolates MHc1 and MHc2 harbored a Asp420-to-Asn substitution at the position corresponding to residue 420 or 426 of the *E. coli* ParE or GyrB, respectively. This ParE substitution was previously described to occur in *M. hominis* mutants obtained in vitro (3) and was shown to be critical for quinolone resistance (10, 19–21). The other ParE sites associated with quinolone resistance (Table 2) in *Staphylococcus aureus* (8, 19, 20), *E. coli* (4), and *Streptococcus pneumoniae* (17) have not been found to be substituted in quinolone-resistant mutants of *M. hominis* selected in vitro or in vivo. Also in three isolates, silent mutations were detected in the codons encoding residues 423, 428, 434, 445, and 447 of the ParE QRDR. Silent mutations found in each of the topoisomerase II genes could be related to a strain-to-strain polymorphism in the nucleotide sequences of these genes.

Concerning the novobiocin susceptibility of the five fluoroquinolone-resistant mutants (Table 1), novobiocin MICs for three isolates, MHa, MHc1, and MHc2, showed a fourfold increase. For these strains, the 5’ region of *gyrB*, including the novobiocin resistance loci described for other species (5, 13), and the homologous 5’ region of *parE* were amplified and sequenced without revelation of any base change compared with the reference strain PG21. A possible active efflux system affecting the novobiocin MIC could be implicated.

Interestingly, isolates MHb1 and MHb2, isolated 6 months apart from the same patient, exhibited identical profiles of resistance to fluoroquinolones, novobiocin, and josamycin, a
16-member macrolide (Table 1); the only difference was in their susceptibility to tetracyclines (doxycycline MIC for MHB2, 8 µg/ml). Strain MHA also showed decreased susceptibility to tetracyclines (Table 1). In the face of the associated tetracycline resistance of these M. hominis isolates, two hypotheses can be considered, one implicating the acquisition of the tetM gene (6) and the other involving an active efflux system (15). Isolates MHc1 and MHc2, isolated at the same time from two different sites in the same patient, have identical antibiotic susceptibility profiles and show the same mutations in QRDRs of their topoisomerase II genes (Table 1). It is tempting to speculate that the paired isolates (MHB1 and MHB2, MHc1 and MHc2) from one patient are closely related or even identical.

In conclusion, the presence of alterations of both the GyrA subunit of DNA gyrase and the ParC or ParE subunit of topoisomerase IV in clinical isolates of M. hominis is in good agreement with our previous studies showing that, in M. hominis mutants selected in vitro, high-level resistance to fluoroquinolones was associated with modifications in both enzyme targets. In a recent study of 20 clinical isolates of E. coli resistant to fluoroquinolones, the failure to detect mutations in the ParE QRDR led the authors to conclude that this region was not linked with the acquisition of quinolone resistance in E. coli clinical isolates (18). In contrast, the finding of a ParE alteration in M. hominis mutants selected in vitro (1, 3) and in vivo (this study), in addition to recent studies describing parE mutants in clinical isolates of S. aureus (19, 20) and S. pneumoniae (17), reinforces the implication of the topoisomerase IV ParE subunit in resistance to fluoroquinolones.

We thank V. Napoli (Laboratoire d’Eylau, Paris, France) for the gift of strains MHB1 and MHB2 and L. Collet (Laboratoire de Bactériologie, Institut Paoli-Calmettes, Marseille, France) for the gift of strains MHc1 and MHc2.

REFERENCES