In Vitro Susceptibilities of *Mycoplasma hominis* to Six Fluoroquinolones as Determined by E Test

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Twenty isolates of *Mycoplasma hominis* were tested for their susceptibility to six fluoroquinolones by the E test. The MICs at which 90% of the isolates were inhibited (in micrograms per milliliter) were as follows: sparfloxacin, 0.031; clinafloxacin, moxifloxacin, and trovafloxacin, 0.063; levofloxacin, 0.25; and ciprofloxacin, 0.5. Increasing the amount of inoculum or incubation in CO₂ elevated MICs by ≤1 dilution. E tests produce fluoroquinolone MICs comparable to those obtained by agar and microbroth dilution for *M. hominis*.

Susceptibility testing of mycoplasmas by traditional methods of broth and agar dilution is nonstandardized and extremely labor-intensive because the required materials are not readily available from commercial sources. We previously showed that the E test, a commercially available plastic strip containing an antimicrobial agent in a continuous gradient, could be used to screen *Mycoplasma hominis* isolates for tetracycline resistance (14). We have now performed further evaluations of the E test with a commercially prepared medium to determine its utility in testing the susceptibility of *M. hominis* to six fluoroquinolones and to determine the effect of the incubation atmosphere and inoculum size on the MICs obtained.

Twenty isolates of *M. hominis* stored frozen at –70°C were thawed, inoculated into PPLO broth (REME, Inc., Lenexa, Kans.), and incubated overnight at 35°C in ambient air. A 1:100 dilution of each culture was prepared in PPLO broth without inhibitors to yield an inoculum of 10⁵ to 10⁷ CFU/ml, subsequently verified by performing serial dilutions and plate counts. This inoculum was shown previously to yield optimum organism concentrations such that the ellipse could be detected (14). Concentrations of <10⁵ CFU/ml produce colonies too sparse to form an ellipse, whereas concentrations of >10⁷ CFU/ml produce colonies that are too small and poorly formed (14).

Volumes of 500 µl of the diluted suspension were pipetted onto 85-mm-diameter SP4 agar plates supplemented with arginine (REME, Inc.), pH 7.7. The plates were rotated to spread the liquid over the entire agar surface and then allowed to dry for 10 min. Streaking plates in the manner recommended by the manufacturer for testing conventional bacteria makes detection of the minute colonies of *M. hominis* much more difficult. E-test strips (containing 0.004 to 32 µg/ml of drug) for ciprofloxacin, clinafloxacin, levofloxacin, moxifloxacin, sparfloxacin, and trovafloxacin were applied according to the manufacturer’s instructions. Two E-test strips were applied to each agar plate. Inoculated plates were incubated in ambient air at 35°C for 72 h. Dienes stain was applied to enhance visualization of colonies under a stereomicroscope. MICs were read as the number on the E-test strip at which colonial growth intersected with the E-test strip. E-test MICs were rounded up to the next highest twofold dilution when necessary for comparative purposes.

MICs at which 50% of the isolates were inhibited (MIC₅₀), MIC₉₀, and ranges are shown in Table 1. Isolates were inhibited by all drugs at concentrations of 0.063 to 0.5 µg/ml. Based on the MIC₉₀, sparfloxacin (0.03 µg/ml) was the most active agent tested, followed by clinafloxacin, moxifloxacin, and trovafloxacin (0.06 µg/ml). MICs determined by E test were comparable to those obtained by agar and/or microbroth dilution in previous studies (also summarized in Table 1) (1, 2, 5–9, 11–13). The in vitro activities, as determined by E test and agar dilution, of one of the agents under investigation in this study, moxifloxacin, for 16 of the 20 isolates of *M. hominis* were also compared directly. These data are reported in detail elsewhere (15). The MIC₉₀ for these isolates, determined by agar dilution, were 0.03 and 0.125 µg/ml, similar to the values obtained by E-test (0.03 and 0.06 µg/ml, respectively).

The effect of incubation of E-test strips in 5% CO₂ on ciprofloxacin MICs was evaluated for 15 isolates, using the same inoculum as that described above. The MIC₉₀ and MIC₅₀ increased by 1 dilution and the pH of uninoculated plates dropped to 7.1 when the strips were incubated in CO₂. The effect of increasing the inoculum size from 10⁵ to 10⁷ CFU/ml had no effect on the MICs for two of five isolates and caused an elevation of the MIC by only 1 dilution in the other three isolates tested.

Susceptibility testing of *M. hominis* to guide patient management is not considered a routine procedure in diagnostic laboratories or reference laboratories. However, this organism is a proven cause of systemic diseases, including wound infections, bacteraemias, abscesses, and bone and joint infections. These types of infections are particularly important in immunocompromised transplant recipients, who may require prolonged antimicrobial chemotherapy (10). Treatment with traditional drugs such as tetracyclines can be compromised by increasing resistance (3). Hence, there is a need for practical and reliable methods of in vitro susceptibility testing. The availability of drugs, such as the fluoroquinolones, that are bactericidal for mycoplasmas (1) makes them attractive agents for use in patients with systemic infections caused by *M. hominis*. Comparison of the in vitro activities of the six agents evaluated in this study demonstrated that the three newest agents, trovafloxacin, moxifloxacin, and clinafloxacin, had...
MICs that were comparable to one another and were only 1 twofold dilution higher than that of the most active agent, sparfloxacin.

Susceptibility testing of mycoplasmas for epidemiological purposes or to establish antimycoplasma activities of new compounds has usually been performed by agar or microbroth dilution. However, diagnostic or reference laboratories may need to test occasional clinical isolates of *M. hominis* to guide antimicrobial therapy for a patient with a primary infection or to assess possible alternative therapeutic options because of treatment failure. In these settings, the ready availability of a method that is cost-effective and simple to perform, allowing multiple drugs to be tested against a single clinical isolate, is important. Current agar dilution and microbroth dilution assays are somewhat lacking in this respect.

Since its development in the late 1980s, the E test has become a popular technique for determining in vitro susceptibilities for organisms for which no other methods have been shown to be practical or reliable (4). This study validates the use of E tests in combination with commercially purchased SP4 agar to determine the MICs of six fluoroquinolones for *M. hominis*, encompassing older compounds that are most active against gram-negative organisms and which possess little antimycoplasma activity, such as ciprofloxacin, as well as newer agents, targeted against gram-positive bacteria, that demonstrate more potency against *M. hominis*. Findings of the present study also demonstrate that *M. hominis* susceptibilities to fluoroquinolones determined by E tests on commercial SP4 agar are comparable to those obtained by other, commonly used methods, even when the medium, pH, inoculum size, and incubation conditions differed. Although care should be taken to use a consistent inoculum to ensure reproducibility, increasing inocula by 2 logs did not change the MICs by more than 1 dilution among the five isolates evaluated.

MICS of many antimicrobial agents, including quinolones, can be affected by testing at a lower pH (13), which may occur during inocula by 2 logs did not change the MICs by more than 1 dilution among the five isolates evaluated.

Susceptibility testing conditions should reflect the physiologic pH (7.2 to 7.4) as closely as possible, unless the growth requirements of the organism preclude this, as in the case of ureaplasmas. The commercial SP4 agar supplemented with arginine, pH 7.7, used in this evaluation was not formulated specifically for susceptibility testing. However, it is unlikely that the higher pH greatly influenced quinolone MICs, since values obtained with other media, usually at a lower pH, gave very similar results (1, 2, 5–9, 11–13). We conclude that the E test is the most practical method for testing the susceptibility of *M. hominis* to tetracyclines and fluoroquinolones in diagnostic laboratories, particularly those not specializing in mycoplasma detection.

AB BIODISK, Solna, Sweden, provided moxifloxacin E tests. REMEL, Inc., Lenexa, Kans., provided media for culture and susceptibility tests. Shawn Banks provided technical assistance.

### REFERENCES


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**TABLE 1. Summary of E-test MICs determined for 20 isolates of *Mycoplasma hominis* and comparison with published MICs for this organism obtained by agar and microbroth dilution**

<table>
<thead>
<tr>
<th>Drug</th>
<th>E test MIC</th>
<th>Broth dilution</th>
<th>Agar dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.063–0.5</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Clinafloxacin</td>
<td>0.008–0.06</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.013–0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.008–0.125</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>≤0.004–0.03</td>
<td>0.016</td>
<td>0.03</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>≤0.004–0.03</td>
<td>0.016</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* MIC ranges and MIC<sub>50</sub> for *M. hominis* from previously published studies are shown to validate the comparability of MICs determined by E test. Numbers in parentheses indicate references from which data were obtained.

* NA, data not available.
