Frequency of selection of fluoroquinolone-resistant mutants of Neisseria gonorrhoeae exposed to gemifloxacin and four other quinolones

Joaquim Ruiz, Angels Jurado, Elena Garcia-Méndez, Francesc Marco, Lorenzo Aguilar, M. T. Jiménez de Anta and Jordi Vila

Introduction

Quinolones have been employed to treat infections due to Neisseria gonorrhoeae, particularly for penicillin-resistant strains. However, during recent years an increase in the number of clinical isolates of N. gonorrhoeae with a decreased susceptibility to these antibacterial agents has been reported. Moreover, development of resistance during treatment with fluoroquinolones and therapeutic failure have been described.

In vitro studies to establish the potential of different quinolones to select resistant mutants have been conducted on various microorganisms. In a series of 10 Gram-negative and Gram-positive microorganisms, not including N. gonorrhoeae, it has been shown that gemifloxacin has a low level of selection of resistant mutants, which is even lower than that of ciprofloxacin or trovafloxacin. Gemifloxacin is a novel fluoroquinolone that possesses enhanced activity against both Gram-negative and Gram-positive microorganisms. Previous studies have shown that it has excellent activity against N. gonorrhoeae. However, the role of this quinolone in the selection of resistant mutants of N. gonorrhoeae remains unknown.

Materials and methods

Microorganisms

Four clinical isolates of N. gonorrhoeae analysed previously were used for the study. Two were resistant to nalidixic acid by virtue of a single mutation in codon 91 of the gyrA gene (Ser→Leu and Ser→Phe, respectively), whereas the other two were susceptible to nalidixic acid with no detectable alteration in either the gyrA or parC gene.

Antimicrobial susceptibility testing

The MICs of gemifloxacin (SmithKline Beecham Pharmaceuticals, Harlow, UK), levofloxacin (Hoechst Marion...
Rousell, Romainville, France), ciprofloxacin, moxifloxacin (Bayer, Leverkusen, Germany) and trovafloxacin (Pfizer Ltd, Sandwich, UK) were determined by an agar dilution method in accordance with the guidelines of the NCCLS.10

Escherichia coli ATCC 25922, N. gonorrhoeae ATCC 49226 and Staphylococcus aureus ATCC 29213 were used as control organisms.

**Determination of spontaneous single-step resistance rates**

The determination of spontaneous single-step resistance rates was performed as described previously,4 with the modification that the isolates were grown on GC-agar and then resuspended in saline solution before inoculation on to GC-agar containing the selected antibacterial agent at 2 ×, 4 × and 8 × MIC. The plates were read at 24 and 48 h. The frequency of spontaneous resistance selected by each fluoroquinolone was calculated as the ratio of the number of bacteria growing divided by the number of bacteria in the original inoculum. The strains that did not appear on the plates were given a frequency of resistance reciprocal to that of the inoculum. The experiments were repeated three times.

**Stability of the resistance**

To establish the resistance stability of mutants, a representative mutant colony from each concentration of antibacterial agent was subcultured onto plates without antibacterial agent prior to MIC determination.

**Analysis of the mutations present in the gyrA and parC genes**

A fragment of the gyrA and parC genes containing the so-called ‘quinolone resistance-determining region’ was amplified using previously described primers and conditions.2,8

**Results and discussion**

The susceptibility and genetic characteristics of the wild-type quinolone-susceptible strains and strains having a previous mutation in the gyrA gene used in this study are shown in Table 1. Mutants able to grow at 2 ×, 4 × or 8 × MIC were obtained with all quinolones tested, except gemifloxacin (Table 2). This finding is similar to those previously reported,2 which indicated that gemifloxacin selected resistant mutants at a lower frequency in Gram-negative and Gram-positive microorganisms, not including N. gonorrhoeae, compared with ciprofloxacin and trovafloxacin. In order to establish the frequency of mutation, all the colonies that grew on antibiotic-containing agar were considered as mutants, regardless of their MIC stability. The frequency of mutation with the quinolones analysed was low. Only when strain 97-692 was grown on agar containing trovafloxacin at 2 × MIC, and when strain 3174 was grown on ciprofloxacin at 2 × and 4 × MIC was the frequency of mutation in the order of 10⁻⁷. No differences were found when the plates were read at 24 and 48 h.

Only one strain (3174) selected at 4 × MIC of trovafloxacin presented a stable increase in its MIC from 0.06 to 0.25 mg/L of this antibacterial agent alone, i.e. did not present alterations in the MICs of other quinolones. The fact that strains able to grow in a medium with a concentration of an antibacterial agent above its MIC do not present a stable increase in the MIC of this antibacterial agent may be due to a temporary induction of some mechanism(s) of quinolone resistance, such as overexpression of some efflux

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**Table 1. Genetic characteristics of the selected isolates**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/L)</th>
<th>GyrA</th>
<th>ParC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAL</td>
<td>CIP</td>
<td>TVA</td>
</tr>
<tr>
<td>Wild type</td>
<td>2.0</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>2815</td>
<td>0.5</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>97-692</td>
<td>64.0</td>
<td>0.120</td>
<td>0.060</td>
</tr>
<tr>
<td>3173</td>
<td>64.0</td>
<td>0.120</td>
<td>0.060</td>
</tr>
<tr>
<td>3174</td>
<td>64.0</td>
<td>0.120</td>
<td>0.060</td>
</tr>
</tbody>
</table>

NAL, nalidixic acid; CIP, ciprofloxacin; TVA, trovafloxacin; LVX, levofloxacin; MOX, moxifloxacin; GEM, gemifloxacin.
Table 2. Frequency of mutation of five quinolones at three different concentrations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum (cfu/mL)</th>
<th>Antibiotic strength</th>
<th>Mutation frequency(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>gemifloxacin</td>
</tr>
<tr>
<td>2815 (Nal S)</td>
<td>3.1 (\times) 10(^{-9})</td>
<td>2 (\times) MIC</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>97-692 (Nal S)</td>
<td>1.0 (\times) 10(^{-9})</td>
<td>2 (\times) MIC</td>
</tr>
<tr>
<td></td>
<td>3173 (Nal R)</td>
<td>5.0 (\times) 10(^{-9})</td>
<td>2 (\times) MIC</td>
</tr>
<tr>
<td></td>
<td>3174 (Nal R)</td>
<td>2.9 (\times) 10(^{-9})</td>
<td>2 (\times) MIC</td>
</tr>
</tbody>
</table>

\(^a\)Mean of three experiments.

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References


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