Elimination of Plasmids by New 4-Quinolones

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Nalidixic acid and six of the new 4-quinolones eliminated F'\text{lac} and various native R plasmids from \textit{Escherichia coli} at one half or one quarter the MIC. Four of eight plasmids tested were cured by all derivatives, with frequencies from 10 to 98%. Quinolones did not eliminate all plasmids that were cured by novobiocin, and vice versa.

A variety of compounds (acridine dyes, ethidium bromide, rifampin) have been reported to eliminate plasmids (1, 4, 16). Recently, investigations of this phenomenon have focused on the coumarine antibiotics novobiocin, coumermycin A1, and clorobiocin (5, 9, 17, 21, 25, 26), known inhibitors of subunit B of DNA gyrase (12), an enzyme responsible for the introduction of negative supercoils into DNA (11). Inhibitors of subunit A of the gyrase, the 4-quinolones (20), have not yet been widely studied with respect to their curing properties, and conflicting reports are known. The eliminating effect of nalidixic acid was reported several years ago (3, 14, 23), but recent investigations could not detect any curing effect of nalidixic and oxolinic acids (5, 9, 17, 21). We report here that nalidixic acid and the new fluorinated 4-quinolones eliminated various native plasmids from \textit{Escherichia coli}.

The bacterial strains used are listed in Table 1. E. coli cells harboring plasmids were incubated at 37°C without shaking in Iso-Sensitest broth supplemented with antibiotics to ensure plasmid presence and diluted to about 500 cells per ml in the same medium without antibiotics. Graded concentrations (one, one-half, one-quarter, one-eighth the MIC) of eliminating agents were added to a total volume of 0.1 ml. Growth occurred for 48 h at 37°C without shaking. Cultures grown at one-half or one-quarter the MIC were diluted appropriately to determine titer. The fraction of plasmid-free cells was determined in two ways. Parallel to viable count measurement, a sample was plated on antibiotic-containing agar to determine the titer of the remaining plasmid-containing cells. The difference between the numbers of colonies on antibiotic and antibiotic-free agar was expressed as percentage of the numbers of colonies on the latter (13). In addition, for each R\textsuperscript{+} strain and each drug tested, 96 colonies grown on antibiotic-free agar were picked at random and inoculated into microtiter plates filled with nutrient broth. Clones were then spot tested on antibiotic agar by using a replicator with 96 pins. Elimination of F'\text{lac} was determined by counting yellow colonies on china-blue lactose agar, indicating absence of lactose fermentation.

Each curing frequency was determined at one-half the MIC or at one-quarter the MIC when growth at one-half the MIC was too poor, because little cell growth is known to reduce elimination frequencies (16; F. E. Hahn, Proc. 13th Int. Congr. Chemother., p. 52/15–16, 1983). Cell titters in control cultures ranged from $6 \times 10^6$ to $4 \times 10^9$/ml (20 to 23 generations of growth), and in treated cultures they ranged from $1 \times 10^4$ to $5 \times 10^9$/ml (11 to 23 generations of growth), indicating suboptimal to optimal growth.

The results of plasmid elimination measured by spot testing are shown in Table 2. Four of the eight plasmids tested were eliminated by all the 4-quinolones, with maximal frequencies of 66 to 98%. The most efficient elimination was obtained with Rts1, which was cured with a frequency of 98% by enoxacin, about 50% by norflaxacin and pipemidic acid, and about 30% by pefloxacin, ofloxacin, and ciprofloxacin. Good curing rates were also obtained with F'\text{lac}; here, enoxacin (66%) and ciprofloxacin (50%) had the highest frequencies. Elimination of R446b and R16 was moderate; curing rates generally did not exceed 30%. Plasmid pBR322 had poor curing frequencies with the quinolones. Unaffected plasmids were pBP1, R27, and R391. Novobiocin, an agent known to eliminate various plasmids (17, 19, 26), was used as a control for the test system. In contrast to the quinolones, it eliminated pBR322, R446b, and R16 to a high extent, but the other plasmids were unaffected. Control cultures showed that spontaneous loss of plasmids was low, with frequencies of 0 to 9%.

Plasmid elimination determined by colony counts on antibiotic-free and antibiotic-containing agar confirmed the results of spot testing of single colonies only when high curing rates were obtained. For low curing rates, differences in the number of cells in two samples and a possible reduction of regrowth rate on antibiotic-containing agar were wrongly interpreted as elimination. In our opinion, spot testing of single colonies is more reliable than counting colonies.

These results confirmed early reports about the elimina-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Inc group</th>
<th>Resistance markers$^{*}$</th>
<th>Reference</th>
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<tr>
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<td>Tc Ap</td>
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<td>J</td>
<td>Km</td>
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<td>H</td>
<td>Tc</td>
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$^{*}$ Antibiotics and concentrations used in liquid and solid media (\mu g/ml): ampicillin (Ap), 50; kanamycin (Km), 10; streptomycin (Sm), 10; sulfonamide (Su), 500; tetracycline (Tc), 10.
tion of plasmids by nalidixic acid (3, 14, 23), although subsequent investigations did not reveal any curing effect of nalidixic acid on plasmids Col V (5), pMB9 (9), pMG110 (17), and pDT4 (21). Most recently, new quinolones have been reported to cure plasmids (S. A. Sonstein and J. B. Courtright, 4th Medit. Congr. Chemother., abstr. no. 705, p. 403, 1984; V. Ucelli and Y. Michel-Briand, Proc. 14th Int. Congr. Chemother., abstr. S-47-11, p. 192, 1985). Our investigations extend these findings. All new 4-quinolones exhibited the eliminating property, although they did so with different frequencies. It seems important to emphasize that, with the exception of pBR322, all were wild-type plasmids.

We do not want to stress the individual elimination frequencies. When we repeated the experiments, the results were generally reproducible, but the error in determining the elimination frequency reached 20 to 50%. This could have been due to the fact that elimination takes place in an extremely narrow range of concentration, which is less than one twofold dilution step, as is known for novobiocin (17). Very little change in concentration could thus vary curing frequency to a great extent. As our experiments were done only at a single concentration, the dependence of elimination on the concentration of various quinolones is being investigated further.

To establish that the elimination of antibiotic resistance markers represents plasmid loss, crude lysates of both plasmid-containing and cured E. coli J53 (Rts1) and W3110 (F' lac) were prepared and run by agarose gel electrophoresis by the method of Hansen and Olsen (15). In control cells a single plasmid band could be observed that was absent in the cured derivatives.

The observed plasmid elimination could represent an enrichment of spontaneously occurring plasmid-free cells owing to faster growth (25) or higher MICs (8). Growth kinetics of J53, J53(Rts1), W3110, and W3110(F' lac) at the drug concentrations tested in the elimination experiments were measured by viable count. The doubling times were 42, 46, 42, and 43 min, respectively. MICs of the same strains measured for enoxacin and ofloxacin were equal. We interpret these results to indicate that plasmid elimination occurs from plasmid-containing cells and that overgrowth of R- cells has minor significance, if any.

Some plasmids could not be eliminated by the quinolones (pBR322, pBP1, R391, R27). The possibility cannot be excluded that this reflects a property of the host strain, but it seems unlikely that pBR322, for example, is extremely stable in strain C600 under quinolone stress and yet is cured by novobiocin. These results may have been due to differences in the molecular mechanisms of action for quinolones and coumarins, but the reasons remain unknown.

The elimination of plasmids at quinolone concentrations still permitting cell growth led us to the conclusion that plasmid replication is more sensitive to quinolone antagonism than is chromosome replication. The elimination of plasmids by novobiocin is explained by the antagonism of subunit B of DNA gyrase (21, 25). It is tempting to relate the curing capacity of the 4-quinolones to the inhibition of subunit A of the gyrase, but the possibility cannot yet be excluded that plasmid elimination by the 4-quinolones reflects an action on a target other than gyrase.

**LITERATURE CITED**


