Activities of Newer Fluoroquinolones against *Shigella sonnei*

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The activities of six fluoroquinolones were determined for 117 separate strains of *Shigella sonnei*. The order of increasing activity (MICs for 90% of strains tested) was enoxacin (0.25 μg/ml), temafloxacin (0.832 μg/ml), ciprofloxacin (0.008 μg/ml), CI-960 (0.006 μg/ml), and PD-131628-2 (0.006 μg/ml). These data, along with results of killing and mutational rate studies, showed that all six fluoroquinolones were highly inhibitory against *S. sonnei* and five fluoroquinolones were rapidly and persistently bactericidal.

Antibiotic treatment is usually indicated for individuals with moderate or severe symptoms of shigellosis (7). The traditionally used therapeutic agents, such as ampicillin and trimethoprim-sulfamethoxazole, have lost much of their effectiveness over the last decade because of increasing multiple antibiotic resistance of shigellae because of their conjugative resistance plasmids (2, 5, 15). The fluoroquinolones are attractive agents for therapy of bacterial enteric diseases for a variety of reasons. They are highly active against many members of the family Enterobacteriaceae (11, 17) and achieve high fecal levels (16); they also have good intracellular and bowel wall penetration (14). Fluoroquinolones act at sites different from those at which other antimicrobial agents act, and resistance to them has not been associated with resistance plasmids (6). Among members of the family Enterobacteriaceae, *Shigella* spp. are one of the leading causes of diarrhea. The most prominent species, *Shigella sonnei*, now constitutes up to 90% of Shigella isolates in industrialized countries (8, 12, 19) and may also serve as a barometer of acquisition and expression of the antimicrobial resistance (5). In the study described here we assessed the in vitro activities of six fluoroquinolones against 117 strains of *S. sonnei* from several countries in North America, Europe, Africa, and Asia. The activities of three new investigational quinolones (CI-960, PD-131628-2, and sparfloxacin) were compared with those of three marketed fluoroquinolones (ciprofloxacin, enoxacin, and temafloxacin).

One hundred seventeen strains of *S. sonnei* were collected from various locations, as follows: 78 from different regions of the United States; 27 from Bulgaria; 4 from Mexico; and 2 each from Guatemala, Egypt, Thailand, and West Africa. *S. sonnei* ATCC 92920, *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

Plasmid analysis was performed by the method of Birnboim and Doly (4). Agarose gel electrophoresis was performed on a horizontal slab gel with 0.7% agarose as described previously (9). The following antimicrobial agents were provided by the indicated suppliers: CI-960, enoxacin, PD-131628-2, and sparfloxacin (Parke-Davis, Ann Arbor, Mich.); ciprofloxacin (Miles Inc., West Haven, Conn.); and temafloxacin (Abbott Laboratories, North Chicago, Ill.). All of the isolates were first studied for susceptibility by the disk diffusion method with a battery of antimicrobial agents (3). The strains were next tested in duplicate for their susceptibilities to the quinolones listed above by an agar dilution technique. Serial two-fold dilutions of the agents were incorporated into Mueller-Hinton agar kept at 50°C, and agar was immediately poured into the plates. The isolates were sub-cultured from MacConkey agar plates into 5.0-ml volumes of Mueller-Hinton broth and were then incubated at 37°C overnight. These broth samples were inoculated onto the antibiotic-containing plates by using a Steers replicator device (18). An inoculum of approximately 10⁶ organisms per spot was used, and the MIC of an agent was the lowest concentration at which visible growth of an isolate was not present. When there were discrepancies between the duplicate results, the higher MIC was chosen. For all antimicrobial agents used, time-kill curves were determined with three selected strains. The strains were grown for 24 h at 37°C in 5.0 ml of Mueller-Hinton broth, and 0.5-ml samples were used to inoculate Erlenmeyer flasks (500 ml) containing 4× the MICs of the test antimicrobial agents in 100 ml of Mueller-Hinton broth. For time-kill studies, an initial concentration in broth of 10⁶ CFU/ml was used. These flasks, along with a control flask to which no test drug was added, were placed in a gyrating incubator at 37°C. Quantitative bacterial counts after 0, 2, 4, 8, and 24 h of exposure to the agents were performed by spreading 0.1 ml of the appropriate dilutions onto Mueller-Hinton agar plates which were

### TABLE 1. Antibiotic susceptibilities of 117 S. sonnei strains to six fluoroquinolones

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (μg/ml)*</th>
<th>Range 50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-960</td>
<td>0.002-0.016</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.004-0.016</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Enoxacin</td>
<td>0.125-0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>PD-131628-2</td>
<td>0.004-0.008</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>0.004-0.016</td>
<td>0.008</td>
<td>0.016</td>
</tr>
<tr>
<td>Temafloxacin</td>
<td>0.016-0.25</td>
<td>0.032</td>
<td>0.032</td>
</tr>
</tbody>
</table>

* 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.
then incubated at 37°C for 24 h before counting. Mutation rates were calculated in the following manner. Cultures of six selected strains were grown in 10 ml of Mueller-Hinton broth at 37°C for 18 to 22 h, centrifuged, and suspended in 2 ml of phosphate-buffered saline. Samples (0.1 ml) were spread onto agar plates containing various concentrations (0.016 and 0.1 pg/ml) of antimicrobial agents, incubated for 48 h at 37°C, and read at 24 and 48 h. Colonies which grew were picked and reidentified as Shigella spp. Development of resistance was confirmed by determination of MICs. The mutation rate was considered to be the ratio of the number of the resistant colonies over the total microbial count (15).

The 117 strains of S. sonnei studied showed diverse antibiograms and plasmid patterns, and there was no evidence of epidemiologic relatedness among the strains. Thus, they were considered separate isolates. Table 1 summarizes the range of MICs and the MICs of the fluoroquinolones for 50 and 90% of the strains studied. All strains were inhibited by the six agents at concentrations of ≤0.25 µg/ml. The order of increasing activity as the MIC for 90% of strains tested was enoxacin (0.25 µg/ml); temafloxacin (0.032 µg/ml); sparfloxacin (0.016 µg/ml); and CI-960, ciprofloxacin, and PD-131628-2 (all 0.008 µg/ml). The rates of killing for the six agents tested against three S. sonnei strains from different locations are shown in Fig. 1. CI-960 showed the most rapid bactericidal effect. Four of the agents (ciprofloxacin, enoxacin, sparfloxacin, and temafloxacin) produced a 3-log unit reduction of the initial inoculum after 4 h; this reduction continued to 24 h. With PD-131628-2 at 24 h, there was overgrowth of resistant mutants of two of the strains (Fig. 1A and C). The MICs for the mutants were 0.25 µg/ml, as determined in duplicate experiments. No mutants were selected on agar containing 10× the MICs of the six agents. At 4× the MICs, low rates of mutation were observed for the following three quinolones: PD-131628-2 (1 × 10⁻⁸), sparfloxacin (1 × 10⁻⁸), and temafloxacin (5 × 10⁻⁸). MIC testing revealed that the mutants had decreased susceptibilities of no more than 4× the MICs of all six agents for the parent strain. The plasmid profiles of all mutants showed the same plasmid pattern that was present in the parent strains. Antimicrobial resistance for Shigella spp. has reached a critical level, particularly because of the multiple resistance genes carried by conjugal plasmids (15, 19). The fluoroquinolones that we tested displayed excellent activities against diverse S. sonnei strains and should become an option for therapy of bacterial dysentery caused by this species. Additionally, our findings suggest that for a group of strains with multiple antimicrobial resistance determinants, practically none were resistant to the quinolones tested. Their rapid and persistently bactericidal effects and excellent intestinal absorption (10), as well as their high concentration in feces (16), make them suitable for oral therapy of shigellosis. Although resistant mutants were found during the course of the experiments described here, the observed increase in resistance was modest and may or may not be of clinical significance (1). The final answer to this question will be evident only after large numbers of patients with shigellosis have been treated with these drugs.

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REFERENCES


