Susceptibilities of Ampicillin-Resistant Strains of *Salmonella* Other Than *S. typhi* to 10 Antimicrobial Agents

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Ampicillin-resistant strains of *Salmonella* other than *S. typhi* constitute a health problem. We tested the antimicrobial susceptibilities to 10 antibiotics of 57 of these strains isolated in a 30-month period. The rates of resistance were as follows: chloramphenicol, 40.3%; tetracycline, 33.3%; gentamicin, 5.3%; co-trimoxazole, 5.3%; nalidixic acid, 1.8%; and amoxicillin-clavulanic acid, cefotaxime, ceftiraxone, aztreonam, and ciprofloxacin, 0%. In our experience, there are alternative antibiotics with excellent in vitro activities.

Salmonellosis is an important health problem worldwide. In recent years, there has been a steady increase in the incidence of salmonella infections reported in the western hemisphere (6, 10). These usually lead to gastrointestinal illness, for which antibiotic therapy is contraindicated (4). However, systemic infections require effective antimicrobial therapy (9). The increasing numbers of neoplasias, collagenoses, hemolytic processes, and AIDS cases are accompanied by increasing numbers of bacteremias and systemic infections caused by strains of *Salmonella* other than *S. typhi* (28).

Ampicillin is one of the agents of choice for systemic salmonellosis because of its excellent in vitro and in vivo activities and its low cost. However, high rates of resistance to ampicillin have been reported in several countries (2, 7, 11, 18, 19, 27). As a consequence, the antimicrobial activities of alternative agents should be monitored in all areas with high incidences of ampicillin resistance. We tested the antimicrobial susceptibilities to 10 antimicrobial agents of 57 ampicillin-resistant (as screened by the agar diffusion method) strains of *Salmonella* spp.

**Bacteria.** Fifty-seven *Salmonella* strains isolated from different patients between May 1988 and December 1990 were used. They were identified by biochemical and serological tests on the basis of standard criteria (16). The species (typed at the National Reference Center for Salmonella, Majadahonda, Madrid, Spain) included 33 strains of *S. enteritidis*, 14 of *S. typhimurium*, 3 of *S. virchow*, 2 of *S. panama*, 2 of nontyped *Salmonella* spp., 1 of *S. bredeney*, 1 of *S. brandenburg*, and 1 of *S. heidelberg*. The strains were kept frozen in skim milk at −30°C. The sources of the strains included stools, blood, and other sites. Organisms were thawed and subcultured on blood agar prior to being tested.

**Antibiotics.** Antibiotics were obtained from their manufacturers as laboratory powders of defined potency and were reconstituted in their recommended diluents to yield stock solutions that were kept frozen. Ampicillin, amoxicillin, and clavulanic acid (Beechem), cefotaxime (Hoechst), ceftiraxone and co-trimoxazole (trimethoprim-sulfamethoxazole, 1:19) (Roche), ciprofloxacin (Bayer), gentamicin (Schering), nalidixic acid (Sigma), aztreonam (Squibb), and tetracycline and chloramphenicol (Antibioticos SA) were tested.

**Susceptibility testing.** All MICs were determined by the agar dilution method with Mueller-Hinton agar (Oxoid) containing graded concentrations of antibiotics and inocula of ca. 10⁸ cells per spot, applied with a Steers replicating device (21). The procedures for susceptibility testing, the definition of the MIC, the use of control strains, and the range of interpretive categories of susceptibility for each antimicrobial agent were those recommended by the National Committee for Clinical Laboratory Standards (21).

Table 1 summarizes the MIC ranges, MICs for 50% of strains (MIC₅₀), and MIC₉₀ of the antibiotics tested. All the strains were found resistant to ampicillin (MIC, >16 µg/ml) when tested by the agar dilution method.

The rates of resistance to each antibiotic tested (breakpoints were as outlined by the National Committee for Clinical Laboratory Standards in its 1991 manual [21]) were as follows: chloramphenicol, 40.3%; tetracycline, 33.3%; gentamicin, 5.3%; co-trimoxazole, 5.3%; nalidixic acid, 1.8%; and amoxicillin-clavulanic acid, aztreonam, cefotaxime, ceftiraxone, and ciprofloxacin, 0%.

Resistance to four of the antibiotics tested (including ampicillin) occurred in 1 isolate (1.8%), and that to three of the antibiotics tested (including ampicillin) occurred in 18 isolates (31.6%). The most common pattern of resistance involved resistance to ampicillin, tetracycline, and chloramphenicol and was observed in 16 isolates (28.1%).

Resistance to ampicillin in *Salmonella* spp. constitutes an increasing problem in the choice of treatment for *Salmonella* infections that need antibiotic therapy. Of particular concern are strains that have acquired simultaneous resistance to other agents besides ampicillin.

In our area, one of every four isolates showed resistance to ampicillin in the study period (1988 to 1990). In other areas, this frequency is higher, reaching as much as 85% (2, 11, 19, 22, 27). In a nearby area, ampicillin resistance occurred in 46% of the isolates (2). Tetracycline and chloramphenicol resistances were very frequent among the isolates studied and were in many cases associated. Other authors have located these resistances in the same plasmid (3). The frequencies obtained in this study contrast with those obtained for a sample of 59 ampicillin-resistant *Salmonella* isolates obtained in 1989 from an area of Madrid ca. 50 km from our hospital. Many of these isolates were susceptible to tetracycline and chloramphenicol (2). There have been reports in the literature of very low rates and of very high rates of resistance to these agents. In some cases, resistances to tetracycline and chloramphenicol are associated and are transferable (11, 14, 19).

All the strains tested were susceptible (72%) or moder-
TABLE 1. Susceptibilities to 10 antimicrobial agents of 57 ampicillin-resistant Salmonella spp.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>4/2–16/8</td>
<td>8/4</td>
<td>16/8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.03–0.5</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.03–0.25</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0.06–0.5</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤1–16</td>
<td>2</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>4–16</td>
<td>4</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤0.25–8</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>≤0.5/9.5–2/38</td>
<td>≤0.5/9.5</td>
<td>≤0.5/9.5</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>4–64</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.015–0.25</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Atelie susceptible (28%) to amoxicillin-clavulanic acid. Our MIC50 and MIC90 were the same as those recently reported in Hong Kong for a sample of 119 ampicillin-resistant Salmonella strains (18). However, these authors considered 25% of their strains resistant to ampicillin-clavulanic acid on the basis of the low breakpoint recommended by the Working Party of the British Society for Antimicrobial Chemistry.

Co-trimoxazole showed very good in vitro activity, although some reports considered it toxic for some patients with AIDS (13), an important group with systemic Salmonella infections.

Ciprofloxacin, aztreonam, and the two extended-spectrum cephalosporins tested showed excellent in vitro activity. This activity proved to be the same as that reported by other authors (18). No resistance was observed, and we consider these agents to be very good alternatives for clinical treatment. However, resistance to extended-spectrum cephalosporins (3, 5, 20, 25) and resistance or diminished susceptibility to quinolones (8, 12, 23, 24) have been described for salmonellae, and this fact is of concern for public health. The genes encoding the new plasmid-mediated β-lactamases with a spectrum of activity extending to include most of the extended-spectrum cephalosporins are derived from point mutations of the genes encoding the common enzymes TEM-1, TEM-2, and SHV-1 (15). TEM-1, TEM-2, and SHV-1 enzymes are very common in our media (26). The fact that a large number of Salmonella strains as well as other strains have these enzymes constitutes a constant danger of the appearance of new extended-spectrum β-lactamases, because it has been demonstrated that the in vivo transfer of genes encoding these enzymes occurs from other species to Salmonella species (3).

On the other hand, because large amounts of the new quinolones are administered in hospitals and to outpatients in Spain (1), we should expect resistance to increase in Salmonella strains. In our study, only one strain showed resistance to nalidixic acid, considered by us and by other authors (17) a reliable marker of fluoroquinolone resistance, and for this strain the MIC of ciprofloxacin was 0.25 µg/ml, ca. 10 times higher than the MIC50 of this antibiotic for the remainder of the strains studied.

We conclude that in our area, the problem of resistance of Salmonella spp. to antibiotics is serious. However, there are agents with excellent in vitro activity and clinical efficacy, confirmed in other studies, that could be used as alternative agents. We recommend a judicious use of these potent agents, because in our opinion, the possibility of the emergence of new antimicrobial resistances is high. We consider that exhaustive surveillance of resistance and control of antibiotic usage are important measures for avoiding the selection and spread of resistant strains.

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REFERENCES


