Susceptibility of Salmonellae to Cephalosporins and to Nine Other Antimicrobial Agents

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Received for publication 19 July 1968

Three cephalosporin-related antibiotics and nine other antimicrobial agents were studied for in vitro effectiveness against 54 recently isolated strains of Salmonella. Minimal inhibitory concentrations determined by the plate dilution method demonstrated the following percentages of resistance: ampicillin, 6%; tetracycline, 13%; streptomycin, 52%; sulfadiazine, 94%; cephaloglycin, 96%; and lincomycin, 100%. No strains were resistant to cephalothin, cephaloridine, chloramphenicol, colistimethate, kanamycin, and polymyxin B. The commonest serotype studied, S. typhimurium, showed the greatest antibiotic resistance, with 21% resistant to ampicillin, 36% resistant to tetracycline, and 71% resistant to streptomycin. Cephalothin and cephaloridine were highly effective in vitro but inhibitory concentrations of 20 to 40 \( \mu \)g of cephaloglycin per ml were required for the majority of Salmonella strains.

The majority of Salmonella are susceptible to cephalothin (6, 11, 15, 16, 21) and to cephaloridine (1, 16, 19, 22) but clinical data are meager. These drugs have the disadvantage of requiring parenteral administration. The appearance of an orally absorbable cephalosporanic acid derivative, cephaloglycin, gave impetus to further evaluation of these drugs against a representative sample of Salmonella.

Fifty-four Salmonella strains were studied by plate dilution for in vitro susceptibility to the three cephalosporin drugs. Nine older antimicrobial agents were tested for comparative purposes and to determine the sensitivity patterns of recently encountered Salmonella strains.

Materials and Methods

Source and identification of cultures. The 54 Salmonella strains were isolated between 1965 and 1967 from fecal specimens of infants and children with acute diarrheal disease. If more than one household member had Salmonellae, only one strain was tested. All strains were identified initially in this laboratory. Confirmation and further identification of specific serotypes was carried out by the Texas State Health Department Laboratory. Serotypes are shown in the Table. These are representative of the serotypes commonly encountered in Texas and the United States (12).

Antimicrobial drugs. The following antibiotic reference standards were obtained from the manufacturers: cephaloridine, cephaloglycin, cephalothin (Eli Lilly & Co., Indianapolis, Ind.) lincomycin (Abbott Laboratories, North Chicago, Ill.), and chloramphenicol (Parke Davis & Co., Detroit, Mich.). Commercial preparations of sodium sulfadiazine, tetracycline (Lederle Laboratories, Pearl River, N.Y.), ampicillin, kanamycin (Bristol Meyers Co., New York), colistimethate (Warner-Chilcott Laboratories, New York) polymyxin B, and streptomycin (Chas. Pfizer & Co., Inc., New York) were used.

Preparation of plates. Serial twofold drug dilutions were prepared in Oxoid sensitivity test broth (Colab Laboratories, Inc., Chicago Heights, Ill.). These were added to Oxoid sensitivity test agar (Colab) at 48 C to obtain final concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.312 \( \mu \)g/ml in agar. In addition, higher concentrations of 80, 160, 320 and 640 \( \mu \)g of sulfadiazine were prepared per ml.

Twenty ml of agar was poured into each plate and incubated overnight at 37 C to check for sterility. All plates were sealed, stored at 4 C, and used within 1 week after preparation. Cephaloglycin plates were used within 24 hr.

Preparation of inocula. Dilutions of overnight seed cultures in 1% tryptone water were prepared with a 0.001-ml calibrated platinum loop added to 10 cc of Oxoid broth. This method has been shown to be as accurate as pipette dilution (14). Mean seed culture densities were 1 \( \times \) 10\(^{-4}\). The initial dilution of 10\(^{-4}\) and the further dilution of 10\(^{-2}\) by the inoculating prong of the inocula-replicator apparatus (4) resulted in a final inoculum of 1 \( \times \) 10\(^{-6}\) on the antibiotic plates. Viable colony counts were made of each inoculum dilution by the standard pour plate method. After 16 to 18 hr of incubation at 37 C, results were recorded as complete inhibition (no visible growth), partial inhibition (isolated colonies), or no inhibition (growth comparable to the antibiotic-free
Minimal inhibitory concentration was defined as the least amount of antibiotic concentration (µg/ml) of medium that resulted in no visible growth. Antibiotic resistance was defined as a minimal inhibitory concentration greater than 10 µg/ml.

**RESULTS**

The susceptibility patterns of the 54 strains tested against ampicillin, cephalothin, cephaloridine, and cephaloglycin are shown in Fig. 1. All strains were susceptible to cephalothin and cephaloridine at concentrations of 5 µg/ml, or less. Fifty-one (94%) were susceptible to ampicillin, and the 3 (6%) “resistant” strains had minimal inhibitory concentrations of 20 µg/ml. Only 2 strains (4%) were sensitive to 10 µg of cephaloglycin per ml, 39 (72%) were susceptible to 20 µg/ml, and all were inhibited by 40 µg of cephaloglycin per ml.

All strains were susceptible to 1.25 µg, or less, of colistimethate and polymyxin B per ml, and to 10 µg, or less, of kanamycin per ml (Fig. 2). Only 26 (48%) were susceptible to 10 µg, or less, of streptomycin per ml; 20 (37%) were inhibited by 20 µg/ml, 4 by 40 µg/ml, and 4 strains required inhibitory concentrations greater than 40 µg/ml (Fig. 2).

**Table 1. Serotypes of Salmonella studied compared with their frequency in Texas and in the entire United States**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Study strains</th>
<th>Percentage of total strains, 1966&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per cent</td>
</tr>
<tr>
<td>S. anatum</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>S. blockley</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>S. cerro</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>S. cholerae suis</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>2</td>
<td>3.7</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>S. infantis</td>
<td>2</td>
<td>3.7</td>
</tr>
<tr>
<td>S. javiana</td>
<td>4</td>
<td>7.4</td>
</tr>
<tr>
<td>S. kentucky</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>S. montevideo</td>
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<td>5.6</td>
</tr>
<tr>
<td>S. muenchen</td>
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<td>S. newport</td>
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<td>18.5</td>
</tr>
<tr>
<td>S. oranienburg</td>
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<td>5.6</td>
</tr>
<tr>
<td>S. panama</td>
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<td>1.9</td>
</tr>
<tr>
<td>S. paratyphi B</td>
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<tr>
<td>S. sandiego</td>
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<td>S. senftenberg</td>
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<tr>
<td>S. typhi</td>
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</tr>
<tr>
<td>S. typhimurium</td>
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<td>25.9</td>
</tr>
<tr>
<td>S. urbana</td>
<td>1</td>
<td>1.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on total isolations from human sources (976 in Texas and 20,040 in U.S.A.) reported to the U.S. Public Health Service (12).

Tetracycline, chloramphenicol, lincomycin, and sulfadiazine susceptibilities are depicted in Fig. 3. All strains were susceptible to chloramphenicol; 47 (87%) were susceptible to tetracycline. All were resistant to lincomycin. Only three strains (6%) were susceptible to sulfadiazine. Even at concentrations of 640 µg of sulfadiazine per ml, all other strains were resistant. The three sulfadiazine-susceptible strains were *S. typhi* (the only *S. typhi* strains tested).

Several serotypes were represented in the antibiotic-resistant organisms. Of the seven tetracycline-resistant strains, five were *S. typhimurium*, one was *S. newport*, and one was *S. oranienburg*. All three ampicillin-resistant strains were *S. typhimurium*. In the 28 streptomycin-resistant strains, the following serotypes (and number of each) were found: *S. typhimurium* (10), *S. newport* (4), *S. javiana* (4), *S. montevideo* (2), *S. oranienburg* (2), *S. anatum* (1), *S. cholera-suis* (1), *S. heidelberg* (1), *S. infantis* (1), *S. kentucky* (1), and *S. senftenberg* (1).

Of the 14 *S. typhimurium* strains, 21% were resistant to ampicillin, 36% to tetracycline, 71% to streptomycin, and 100% to sulfadiazine, lincomycin, and cephaloglycin. None was resistant to the other antibiotics.

Excluding from consideration sulfadiazine, lincomycin, and cephaloglycin, there were very few multiply-resistant strains. Two strains were
triply-resistant to ampicillin, tetracycline, and streptomycin. Five strains showed resistance to two antibiotics (four to tetracycline and streptomycin and one to tetracycline and ampicillin). The 25 other resistant strains were resistant to a single antibiotic.

**DISCUSSION**

The most effective antibiotics in vitro against the *Salmonella* species tested in this study were chloramphenicol, cephalothin, cephaloridine, kanamycin, colistimethate, and polymyxin B. No strains resistant to these drugs were found.

Arguing from in vitro sensitivity to clinical effectiveness can be hazardous. Kanamycin and the polymyxins exemplify well this difficulty. In this study and in others (2, 13), kanamycin has been highly effective in vitro, but inconclusive results or failure in the treatment of salmonellosis and typhoid fever have been reported (13, 17). Similarly, the unimpressive clinical results (3) coupled with the relatively high toxicity of the polymyxins make these undesirable drugs for therapy of *Salmonella* infections.

There has been little clinical experience with cephalosporins in *Salmonella* infections. Walters, Romansky, and Johnson (21) treated an 85-year-old man with cephalothin for *S. typhimurium* septicemia. Blood cultures became negative, but the patient died of his primary neoplastic disease on the eighth day of therapy. With cephaloridine, Walker (20) and Hermans et al. (5) found little or no benefit in treatment of typhoid fever. Holloway and Scott (8) reported that an adult with *S. derby* infection and an infant with *S. heidelberg* infection both failed to respond to cephaloridine, and subsequently improved with chloramphenicol therapy. Apicella, Perkins, and Saslaw (1) noted marked clinical improvement from cephaloridine in four patients with salmonellosis, but the bacteria were not eradicated in any of the cases.

These clinical reports are too few to serve as a basis for conclusive statements about the efficacy of cephalosporins in *Salmonella* infections. Cephalothin deserves further study in preference to cephaloridine because of the frequency of serious renal toxicity recently reported with the latter drug (7, 9, 18).

The apparent resistance of *Salmonellae* to cephaloglycin may be artifactual because of the rapid deterioration of the drug in solution. To minimize this effect, testing was performed within 24 hr of preparation of the agar plates. The manufacturer states that inhibitory end points must be interpreted after 12 hr of incubation, rather than 18 or 24 hr, based on their tube dilution studies, but we found no difference between 12-hr and 18-hr minimal inhibitory concentra-
tions by the plate dilution method used in these studies. Wick and Boniece (22) tested one strain of S. typhi with cephaloglycin and found inhibitory concentrations of \( \leq 0.39 \) \( \mu \)g/ml at 12 hr and 1.56 \( \mu \)g/ml at 24 hr, using \( 10^3 \) organisms inoculum. With \( 10^2 \) organisms inoculum, the inhibitory concentrations were 0.78 and 6.25 \( \mu \)g/ml, respectively, at 12 and 24 hr. The small inoculum of 100 to 1,000 organisms used in our study dispels any argument that a large inoculum may have overwhelmed the inhibitory ability of this unstable drug in vitro. Furthermore, in unpublished studies performed in this laboratory using identical methodology, J. D. Nelson found minimal inhibitory concentrations less than 5 \( \mu \)g/ml against Shigella strains. For these reasons, we conclude that the concentrations of 20 or 40 \( \mu \)g of cephaloglycin per ml required to inhibit growth of almost all the Salmonellae studied suggest that this drug has dubious potential value for Salmonella infections.

The efficacy or advisability of antimicrobial therapy of simple Salmonella enteritis is disputable, but there is no disagreement about the need for antibiotic treatment of more serious diseases, such as septicemia and meningitis, and of focal disease outside the intestinal tract. Chloramphenicol remains the preferred drug of those antibiotics with proved clinical efficacy in serious Salmonella infections. Ampicillin is also effective, but a significant proportion of S. typhimurium strains are resistant. The higher proportion of S. typhimurium strains resistant to ampicillin (21\%) and tetracycline (36\%) compared to other types of Salmonellae was also noted by Kaye, Merselis, and Hook (10) who found 20\% resistant to these drugs. Because S. typhimurium is the commonest strain isolated in human infections in the United States, ampicillin cannot always be relied on for initiating therapy in serious Salmonella infections. The search for a satisfactory drug alternative to chloramphenicol must proceed. Of the newer drugs, cephalothin appears to offer potential to fill this role, and it deserves further clinical evaluation.

ACKNOWLEDGMENT

John D. Nelson was the recipient of Research Career Development Award 2-K03-Al-11650-06 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED