Influence of Growth Medium on the In Vitro Activities of Second- and Third-Generation Cephalosporins Against Streptococcus faecalis

DANIEL F. SAHM,1†‡ C. N. BAKER,2 R. N. JONES,3 AND C. THORNSBERRY2

Laboratory Program Office, Centers for Disease Control,1 and Center for Infectious Diseases, Hospital Infections Program,2 Atlanta, Georgia 30333, and Kaiser Foundation Hospitals, Portland, Oregon 970153

Received 15 February 1984/Accepted 30 May 1984

The influence of culture medium of the MICs of eight cephalosporins for 45 strains of Streptococcus faecalis was investigated. The MICs of cefalothin, cefamandole, and cefoperazone were not substantially influenced by the type of culture medium used. In contrast, MICs of cefuroxime, ceftizoxime, cefotaxime, cefmenoxime, and ceftriaxone varied markedly with both the commercial brand and the blood content of the broth used. The use of Mueller-Hinton broths (from Oxoid Ltd., GIBCO Diagnostics, and Difco Laboratories) supplemented with 5% lysed sheep blood frequently resulted in MICs that were ≥16 times lower than the MICs obtained with these same broths without blood. Similar, but less marked, patterns were observed when supplemented and unsupplemented brain heart infusion and Sceptor broths were used. The influence of the broth on MICs suggests a complex interaction between some cephalosporins, medium components, and organisms. The cephalosporins that were affected by media share an identical moiety at the 7-acyl position (cefoxime is slightly different), but this structure is not shared by those cephalosporins that were not affected. This commonality in structure at the 7-acyl position may be partially responsible for the observed results.

Numerous in vitro studies have demonstrated that enterococci are generally resistant to newer second- and third-generation cephalosporins such as cefamandole (25), cefuroxime (20, 21), cefoperazone (14), ceftizoxime (7), cefmenoxime (1, 9), ceftriaxone (5, 28), and cefotaxime (8, 13). However, an investigation done in our laboratory has shown that the susceptibility of Streptococcus faecalis to one of these caphalosporins (cefoxaxime) may be substantially influenced by the commercial brand and the blood content of the Mueller-Hinton (MH) agar medium used for susceptibility testing by the standard disk diffusion method (24). The testing of several S. faecalis strains against cefoxaxime often resulted in zone sizes indicative of susceptibility when blood-supplemented MH agar was used, whereas zone sizes indicative of resistance usually resulted when unsupplemented MH agar was employed. In addition, more S. faecalis strains appeared susceptible to cefoxaxime with the use of blood-supplemented MH agar from Oxoid Ltd. (KC Biological Inc., Lenexa, Kans.) and BBL Microbiology Systems (Cockeysville, Md.) than was apparent with the use of supplemented MH agar from other sources. This medium-associated major discrepancy is of obvious clinical importance as well as of major concern with respect to the standardization of in vitro susceptibility testing.

To aid in the understanding of the mechanism(s) responsible for this major discrepancy, we initiated further studies that might clarify and define the in vitro situations in which this phenomenon was observed. The purpose of this present study was twofold. First, we sought to determine whether the medium-associated major discrepancy observed with S. faecalis and cefotaxime by the disk diffusion method could also be demonstrated by the microdilution broth method; second, we sought to determine whether other second- and third-generation cephalosporins would also demonstrate this medium-associated discrepancy when tested against several S. faecalis strains.

MATERIALS AND METHODS

For this investigation, 45 strains of S. faecalis were tested against various first-, second-, and third-generation cephalosporins by the microdilution broth method. These tests were done with broth media of various types and from different commercial sources (see Table 1); each broth was tested with and without 5% lysed sheep blood.

Organisms. Forty-five S. faecalis strains used for this investigation were kindly supplied by Richard Facklam, Reference Bacteriology Section, Respiratory and Special Pathogens Branch, Centers for Disease Control, Atlanta, Ga. Control bacterial strains for susceptibility testing included Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853.

Media. MH broth media were supplied by Oxoid; GIBCO Diagnostics, Madison, Wis.; and Difco Laboratories, Detroit, Mich. The brain heart infusion (BHI) broth used in this study was also supplied by Difco. Sceptor gram-positive broth was graciously donated by George Evans, BBL Microbiology Systems. All broth media were prepared, with or without the addition of 5% lysed sheep blood, according to the supplier’s directions. Lysed sheep blood was prepared by previously described methods (27). In all, 10 different types of broth media were used for the testing of the various cephalosporins against the S. faecalis strains. These media included GIBCO MH broth with and without 5% lysed sheep blood, Oxoid MH broth with and without 5% lysed sheep blood, Difco MH broth with and without 5% lysed sheep blood, BHI broth with and without 5% lysed sheep blood, and Sceptor gram-positive broth (Sceptor broth) with and without 5% lysed sheep blood.

Antibiotics. The cephalosporins tested in this study were received in powdered form and tested at concentrations of 2, 4, 8, 16, 32, and 64 μg/ml in the various types of broth media.

Susceptibility testing: Susceptibility testing of the S. faecalis and control strains with the various cephalosporins was performed by the microdilution broth method recommended by the National Committee for Clinical Laboratory Standards (16). Because specific breakpoints have not been established for some of these cephalosporins, the results were not tabulated in a categorical format (i.e., resistant, intermediate, susceptible), but rather were analyzed strictly on the basis of the observed MICs.

**RESULTS**

We first analyzed the data by determining the number of S. faecalis strains for which we obtained an MIC value of \( \leq 8 \) \( \mu g/ml \) with each cephalosporin in each type of broth medium (Table 1). The number of strains with MICs of \( \leq 8 \) \( \mu g/ml \) for cephalothin, cefamandole, and cefoperazone was generally much less than for the other cephalosporins, and the number of strains for which the MICs of cephalothin, cefamandole, and cefoperazone were \( \leq 8 \) \( \mu g/ml \) did not vary extensively with the brand or blood content of the broth used. The use of unsupplemented Oxoid MH resulted in none of the 45 S. faecalis strains having a MIC of \( \leq 8 \) \( \mu g/ml \) for any of the cephalosporins tested. In comparison to the results obtained with cephalothin, cefamandole, and cefoperazone, the MICs of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime were \( \leq 8 \) \( \mu g/ml \) for a much larger number of S. faecalis strains; in addition, the number of strains for which the MIC values were \( \leq 8 \) \( \mu g/ml \) was closely related to the brand and to the blood content of the broth medium that was employed. Results obtained with the unsupplemented broths revealed some variations in the number of S. faecalis strains for which MIC values were \( \leq 8 \) \( \mu g/ml \). When unsupplemented Oxoid MH broth was used, the MICs of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime were \( \leq 8 \) \( \mu g/ml \) for any of the S. faecalis strains. However, the MICs of these five cephalosporins were \( \leq 8 \) \( \mu g/ml \) for a much larger number of S. faecalis strains when unsupplemented BHI broth was used. The number of strains for which the MICs were \( \leq 8 \) \( \mu g/ml \) did not vary appreciably with the use of unsupplemented GIBCO MH, Difco MH, or Sceptor broths.

Comparisons of the MIC results obtained with the use of blood-supplemented broths did reveal brand-to-brand variations in the number of strains for which the MICs of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime were \( \leq 8 \) \( \mu g/ml \) (Table 1). However, the most notable pattern that was observed was the remarkable increase, with the use of blood-supplemented broths, in the number of S. faecalis strains for which the MICs of these five particular cephalosporins were \( \leq 8 \) \( \mu g/ml \) (Table 1). For each brand of broth used, the number of strains for which the MICs of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime were \( \leq 8 \) \( \mu g/ml \) was greater with the blood-supplemented broth than with the unsupplemented counterpart; a single exception was noted with cefuroxime tested in Difco MH broth. This blood-associated phenomenon was most pronounced with Oxoid MH broth; approximately one-half of the S. faecalis strains tested demonstrated MIC values that were \( \leq 8 \) \( \mu g/ml \) in the presence of lysed sheep blood, whereas none of the strains demonstrated MIC values \( \leq 8 \) \( \mu g/ml \) in the absence of blood. Although not as drastic, a similar pattern was observed when each of the other four blood-supplemented brands was compared with its unsupplemented counterpart. Therefore, the initial analysis of the data provided evidence that both the presence of blood and the brand of broth medium used may influence the in vitro activity of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime against S. faecalis.

To determine the extent to which lysed sheep blood and the brand of broth medium used influenced the MICs of the various cephalosporins, the number of strains for which there was a \( \geq 8 \)-fold and a \( \geq 16 \)-fold decrease in MIC was tabulated for each type of broth used (Table 2). Cephalothin, cefamandole, and cefoperazone each had a \( \geq 8 \)-fold decrease in their MICs for a substantial number of S. faecalis strains when the use of blood-supplemented Oxoid MH was compared with the use of unsupplemented Oxoid MH; this drop in MICs was not observed with the use of any of the other brands of broth. The use of blood-supplemented Oxoid MH broth resulted in only a few strains for which the MICs of

### Table 1. Number of S. faecalis strains with MICs of \( \leq 8 \) \( \mu g/ml \) when tested in various broths with and without blood

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Oxoid MH</th>
<th>GIBCO MH</th>
<th>Difco MH</th>
<th>BHI</th>
<th>Sceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalothin</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefmenoxime</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* A total of 45 strains were tested.

### Table 2. Number of S. faecalis strains with \( \geq 8 \)-fold and \( \geq 16 \)-fold lower MIC when tested in blood-supplemented media than when tested in same media without blood

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Oxoid MH</th>
<th>GIBCO MH</th>
<th>Difco MH</th>
<th>BHI</th>
<th>Sceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalothin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefmenoxime</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* A total of 45 strains were tested.
cephalothin, cefamandole, and cefoperazone decreased ≥16-fold. In contrast, the use of each of the five brands of broth that were supplemented with blood resulted in a ≥8-
fold drop in the MICs of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime for several S. faecalis strains. However, the number of strains for which ≥8-fold decreases in the MICs of these five cephalosporins were observed varied with the brand of broth. The use of blood-
supplemented Oxoid MH broth resulted in the greatest number of S. faecalis strains for which ≥8-fold and ≥16-fold decreases in MICs were observed. Blood-supplemented GIBCO MH and Difco MH broths, respectively, demonstrated the second and third highest number of strains for which the MICs of cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime decreased ≥8-fold and ≥16-fold. The use of blood-supplemented BHI and Sceptor broths resulted in the lowest number of S. faecalis strains for which these substantial MIC decreases were observed.

Because cefuroxime, cefotaxime, cefotaxime, ceftriaxone, and cefmenoxime were the cephalosporins with activities that were most affected by the brand and blood content of the broth used, the distribution of the number of strains that were inhibited by each cephalosporin concentration tested was tabulated according to the broth type employed (Tables 3 through 7).

The data in Table 3 aptly demonstrate the remarkable influence that the brand and blood content of the broth used had on the MIC of cefotaxime for the S. faecalis strains. The use of unsupplemented Oxoid MH, GIBCO MH, and Difco MH broths resulted in the MIC of cefotaxime being ≥64 μg/ml for the vast majority of S. faecalis strains. In striking contrast, the use of these same three broths supplemented with blood resulted in the MIC of cefotaxime being ≥64 μg/ml for only approximately one-half of the S. faecalis strains tested. The use of these three blood-supplemented broths also resulted in a more even distribution of the number of strains inhibited by each concentration of cefotaxime tested than that which resulted with the use of their unsupplement-
ed counterparts. For supplemented and unsupplemented BHI and Sceptor broths, the number of strains for which the cefotaxime MIC of ≥64 μg/ml was obtained was comparable, as was the distribution of the number of strains inhibited by each cefotaxime concentration tested. However, the cefotaxime MIC of ≥2 μg/ml was observed for more strains when BHI broth was used than when Sceptor broth was used.

The MICs of cefuroxime (Table 4), cefuroxime (Table 5),

<table>
<thead>
<tr>
<th>Table 4. Number of S. faecalis strains that gave the designated MIC value when tested against cefuroxime in various broths with and without blood*</th>
<th>No. of strains with designated MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (μg/ml)</td>
<td>Oxoid MH</td>
</tr>
<tr>
<td></td>
<td>B*</td>
</tr>
<tr>
<td>≥2</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>≥64</td>
<td>18</td>
</tr>
</tbody>
</table>

| Table 3. Number of S. faecalis strains that gave the designated MIC value when tested against cefuroxime in various broths with and without blood* |
|---|---|
| MIC (μg/ml) | Oxoid MH | GIBCO MH | Difco MH | BHI | Sceptor |
| | B* | NB† | B | NB | B | NB | B | NB |
| ≥2 | 17 | 0 | 3 | 1 | 4 | 2 | 16 | 11 | 7 | 3 |
| 4 | 3 | 0 | 7 | 1 | 3 | 0 | 2 | 3 | 3 | 1 |
| 8 | 4 | 0 | 5 | 1 | 6 | 2 | 3 | 1 | 4 | 4 |
| 16 | 1 | 0 | 3 | 0 | 2 | 0 | 1 | 3 | 4 | 1 |
| 32 | 2 | 0 | 7 | 1 | 5 | 0 | 4 | 3 | 2 | 3 |
| ≥64 | 18 | 45 | 20 | 41 | 25 | 41 | 19 | 24 | 25 | 33 |

* A total of 45 strains were tested.
† B. Presence of 5% lysed sheep blood.
‡ NB. Absence of 5% lysed sheep blood.

**DISCUSSION**

The results of this investigation have demonstrated that the activity of cefuroxime against numerous strains of S. faecalis, as determined by the microdilution broth method, varied extensively with the blood content and the brand (Oxoid, GIBCO, Difco, BHI, or Sceptor) of broth medium used for testing (Tables 1 through 3). Cefuroxime was least active against S. faecalis strains when Oxoid MH, GIBCO MH, and Difco MH broths without blood supplementation were used. In comparison, the use of BHI broth without blood and, to a lesser extent, Sceptor broth without blood resulted in a greater activity of cefuroxime against the strains of S. faecalis than resulted with the use of the MH broths without blood. Although the activity of cefuroxime did vary with the brand of broth used for susceptibility testing, the most notable variations in cefuroxime activity were associated with the content of 5% lysed sheep blood in the broth media. For each brand of broth used, cefuroxime was more active against a greater number of strains when testing was done in broth containing blood than when testing was done in the unsupplemented broths. These findings are in agreement with our earlier study that demonstrated that the use of blood-supplemented MH agar in the disk diffusion test.
TABLE 5. Number of *S. faecalis* strains that give the designated MIC value when tested against cefuroxime in various broths with and without blood

<table>
<thead>
<tr>
<th>MIC (μg/ml)</th>
<th>Oxoid MH</th>
<th>GIBCO MH</th>
<th>Difco MH</th>
<th>BHI</th>
<th>Sceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B⁵ NB⁴</td>
<td>B NB</td>
<td>B NB</td>
<td>B NB</td>
<td>B NB</td>
</tr>
<tr>
<td>≤2</td>
<td>4 0 1 2</td>
<td>2 1</td>
<td>2 2</td>
<td>2 2</td>
<td>3 2</td>
</tr>
<tr>
<td>4</td>
<td>8 10 0 0</td>
<td>0 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>8</td>
<td>16 2 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>16</td>
<td>32 2 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>32</td>
<td>≥64</td>
<td>17 44 21 42</td>
<td>27 42 21 29</td>
<td>32 36</td>
<td></td>
</tr>
</tbody>
</table>

* A total of 45 strains were tested.
* B, Presence of 5% lysed sheep blood.
* NB, Absence of 5% lysed sheep blood.

resulted in a greater number of *S. faecalis* strains being susceptible to cefotaxime than were susceptible when agar media without blood were used. In addition, these results confirm our previous finding that blood-supplemented Oxoid MH agar was the medium most frequently associated with this phenomenon (24).

Also of interest in this present study is the observation that other cephalosporins were affected, in a manner similar to cefotaxime, by the type of broth medium used for susceptibility testing (Tables 1, 2, and 4 through 7). Cefotaxime (Table 4), cefuroxime (Table 5), cefmenoxime (Table 6), and ceftriaxone (Table 7) were all least active against the *S. faecalis* strains when tested in Oxoid MH, GIBCO MH, and Difco MH broths without blood. As with cefotaxime, the activities of these cephalosporins were greater when tested in BHI and Sceptor broths without blood than when tested in MH broths without blood. As was also noted with cefotaxime, the activities of cefotaxime, cefuroxime, cefmenoxime, and ceftriaxone were more markedly influenced by the presence of 5% lysed sheep blood in the broth medium. Each of these cephalosporins was more active against a greater number of *S. faecalis* strains when blood-supplemented broths were used than when unsupplemented broths were used. In contrast, the activities of cefalothin, cefamandole, and cefoperazone were not extensively influenced by the type of broth medium used for susceptibility testing (Tables 1 and 2).

Our observation that the in vitro activities of certain cephalosporins against *S. faecalis* are markedly influenced by the growth medium used presents new and confounding information with respect to the susceptibility testing of *S. faecalis* with these cephalosporins. Although earlier studies by Washington and Yu (29) and Purisiano et al. (23) have shown that the type of growth medium may influence the susceptibility testing results obtained with cephalosporins, the results of these investigations cannot be accurately compared with those of this present investigation, for various reasons. In those studies the effect of blood supplementation was not studied, the test organisms did not include *S. faecalis*, and, obviously, the newer second- and third-generation cephalosporins were not available for investigation. An earlier study by Brenner and Sherris (3) did report that the type of growth medium used markedly affected the activity of cefalothin against enterococci, but cephalothin was the only cephalosporin tested and only one strain of enterococcus (species not given) was tested. In more recent studies of the in vitro activities of newer cephalosporins such as cefotaxime (19), ceftriaxone (2, 5), cefuroxime (20), and cefotizoxime (7), no significant effects of media on the activities of these cephalosporins were demonstrated. However, these studies were concerned with the activity of cephalosporins against various species of gram-negative bacilli and did not report on the effect of media on susceptibility testing results obtained with strains of *S. faecalis*. In studies with *S. aureus*, Gong et al. (6) and Peterson et al. (22) have reported that the in vitro activities of cefazolin, cephalothin, and cephalaridine were influenced by the growth medium used for susceptibility testing. These investigators thought that the differences were due to medium enhancement of β-lactamase production (6); if this is true, the significance of their results with respect to ours obtained with *S. faecalis*, an organism that does not usually produce β-lactamase, is doubtful.

By demonstrating that the in vitro activities of certain cephalosporins (cefotaxime, ceftriaxone, cefuroxime, cefmenoxime, and ceftriaxone) against *S. faecalis* vary markedly with the type of growth medium used for susceptibility testing, we have raised obvious concerns about the medium

TABLE 6. Number of *S. faecalis* strains that gave the designated MIC value when tested against cefmenoxime in various broth with and without blood

<table>
<thead>
<tr>
<th>MIC (μg/ml)</th>
<th>Oxoid MH</th>
<th>GIBCO MH</th>
<th>Difco MH</th>
<th>BHI</th>
<th>Sceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B⁵ NB⁴</td>
<td>B NB</td>
<td>B NB</td>
<td>B NB</td>
<td>B NB</td>
</tr>
<tr>
<td>≤2</td>
<td>2 0 1 2</td>
<td>2 2</td>
<td>2 2</td>
<td>2 2</td>
<td>3 2</td>
</tr>
<tr>
<td>4</td>
<td>8 10 0 0</td>
<td>0 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>8</td>
<td>16 2 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>16</td>
<td>32 2 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>32</td>
<td>≥64</td>
<td>17 44 21 42</td>
<td>27 42 21 29</td>
<td>32 36</td>
<td></td>
</tr>
</tbody>
</table>

* A total of 45 strains were used.
* B, Presence of 5% lysed sheep blood.
* NB, Absence of 5% lysed sheep blood.
of choice for testing these particular cephalosporins against *S. faecalis* and the true effectiveness of these antibiotics in treating *S. faecalis* infections. An understanding of mechanism(s) involved in this observed phenomenon would be useful in resolving these concerns. Although this mechanism(s) cannot be delineated on the basis of our results alone, certain patterns have been observed that may be useful for the understanding and the eventual resolution of this problem. Classically, the activity of antibiotics is dependent upon inherent characteristics of the organisms and the drugs and is influenced by the environment (media or tissue) in which they interact. The explanation for our unusual results will probably encompass all three of these factors. Inherent characteristics of the organism (*S. faecalis*), the antibiotics (ceftaxime, cefotaxime, cefuroxime, cefmenoxime, and ceftriaxone), and the various environments (different brands of blood-supplemented and unsupplemented broth) all appear to be important factors that contributed to the results obtained in this investigation.

The possibility that certain singular inherent characteristics of *S. faecalis* are at least partially responsible for the results observed is supported by observations made both by us and by other investigators. As part of the present study, using the various types of broth media, we tested *S. aureus*, *E. coli*, and *P. aeruginosa* control strains against each cephalosporin. The results demonstrated that the MICs of cefotaxime, cefmenoxime, cefuroxime, cefazidime, and ceftriaxone for these control strains did not vary from medium to medium by more than one twofold dilution (data not shown). Similarly, results reported by other investigators have failed to demonstrate any effect of growth media on the in vitro effectiveness of these cephalosporins against various gram-negative bacilli (2, 5, 7, 19, 20). Therefore, because the phenomenon observed in the present investigation with *S. faecalis* has not been observed with other bacterial species, it seems likely that inherent characteristics of the *S. faecalis* strains are important contributing factors to the mechanism(s) involved. Another indication that certain singular characteristics of *S. faecalis* may be involved has been provided by Muytjens and van der Ros-van de Repe (14). Although no comparative data were presented, these investigators did discuss the potential for MICs for *S. faecalis* to vary up to 16-fold with different media used for testing.

In addition to characteristics of the microorganisms (*S. faecalis*), certain broth-medium components must also significantly affect the in vitro activities of cefotaxime, ceftriaxone, cefuroxime, cefmenoxime, and ceftriaxone. The decreased activities of these particular cephalosporins in unsupplemented Oxoid MH, Gibco MH, and Difco MH broths, as compared with activities in unsupplemented BHI and Sceptor broths, indicate that broth base constituents may be factors contributing to the observed results. In addition, the increase in activity associated with blood supplementation was more pronounced with Oxoid MH, Gibco MH, and Difco MH media than with BHI or Sceptor broths, further indicating that differences in broth base constituents are factors contributing to the observed phenomenon (Table 2). However, the complex and sometimes undefined nature of the components of these bases and the proprietary interests of the manufacturers concerning these constituents make drawing conclusions about the specific component(s) responsible for these unusual results both difficult and highly speculative.

Although differences in the MICs obtained with the use of various unsupplemented broths did occur, more striking differences in the MICs of the implicated cephalosporins were observed when results obtained with the use of blood-supplemented broths were compared with the results obtained with unsupplemented broths (Table 1). The use of blood-supplemented broth resulted in an increased activity of cefotaxime, cefuroxime, cefotaxime, cefmenoxime, and ceftriaxone against the *S. faecalis* strains. The mechanism by which blood or blood components may influence the in vitro activity of these particular cephalosporins is not understood. Wright and Frogge (30) have demonstrated that in the presence of lysed whole blood, cephalosporins having a 3-acetoxyethyl group may be hydrolyzed by acylases to their desacetyl derivative, resulting in various effects on the cephalosporins' activities. Results obtained in this present study as well as those of other studies offer contradictions to this explanation. In the present study, the activity of cephalothin, a cephalosporin containing a 3-acetoxyethyl group, was not nearly as affected by the presence of blood as were the activities of cefuroxime, cefmenoxime, cefotaxime, and ceftriaxone, none of which contain a 3-acetoxyethyl group (Table 2, Fig. 1). In addition, the activity of cefotaxime, a cephalosporin that is naturally deacetylated at the 3 position, was affected by the presence of blood to an extent comparable to cefotaxime, a cephalosporin that contains a 3-acetoxyethyl group (Fig. 1). Furthermore, Jones et al. (12) and Neu (18) have shown that the in vitro activities of desacetyl-cefotaxime and cefotaxime against *S. faecalis* are comparable. Deacetylation, therefore, does not appear to be the mechanism by which lysed sheep blood affected the in vitro activities of cefotaxime, cefuroxime, cefotaxime, cefmenoxime, and ceftriaxone.

In addition to the factors associated with the *S. faecalis* strains and the broth and blood components that contribute to the in vitro phenomenon observed in this investigation, certain characteristics of the particular cephalosporins are also pertinent to the understanding of the underlying mechanism(s) involved. The activities of cephalothin, cefamandole, and cefoperazone were much less drastically influenced by the type of broth used than were the activities of cefotaxime, cefotaxime, cefuroxime, cefmenoxime, and ceftriaxone (Table 1). Although no absolute explanation for these results can be offered at this time, a closer analysis of the cephalosporins most affected by the broth medium used indicates that certain structural characteristics of these particular antibiotics may contribute significantly to the observed results. Cefuroxime, cefmenoxime, cefotaxime, cefotaxime, and ceftriaxone all share common structures in

---

**TABLE 7. Number of *S. faecalis* strains that gave the designated MIC value when tested against ceftriaxone in various broths with and without blood.**

<table>
<thead>
<tr>
<th>MIC (μg/ml)</th>
<th>No. of strains with designated MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxoid MH</td>
</tr>
<tr>
<td>≤2</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>≥64</td>
<td>15</td>
</tr>
</tbody>
</table>

* A total of 45 strains were tested.
* B. Presence of 5% lysed sheep blood.
* NB. Absence of 5% lysed sheep blood.
their $R_2$ (7-acyl position) moieties (Fig. 1). Specifically, each of these five cephalosporins contains a methoxyimino group at the 7-acyl position that bridges the cephalosporin nucleus with a heterocyclic ring. For cefmenoxime, cefitoxime, cefotaxime, and ceftriaxone the heterocyclic structure is an identical aminothiazolyl ring. Although cefuroxime also contains the methoxyimino group at the 7-acyl position, its heterocyclic ring (a furyl moiety) differs slightly from those of the other four implicated cephalosporins. Whereas these five cephalosporins share common structural features in their $R_2$ moieties, none of them share a common structure in their $R_1$ (3-position) moiety. Additionally, the cephalosporins least affected by the type of broth used (cephalothin, cefamandole, cefoperazone) are strikingly different in the structure of their $R_2$ moieties from the other five cephalosporin (Fig. 1). It is possible that the similarities in structure of the $R_2$ moieties of cefuroxime, cefmenoxime, cefitoxime, cefotaxime, and ceftriaxone may be merely coincidental with respect to being the cephalosporins most affected by the type of broth medium used for susceptibility testing. However, another interesting, but speculative, explanation for this in vitro phenomenon may also be proposed. Georgopapadakou and Liu (10) have demonstrated that a strain of $S.$ faecalis will contain at least six different penicillin-binding proteins, and they have suggested that the resistance of $S.$ faecalis to cephalosporins may be due to a lack of affinity between these penicillin-binding proteins and cephalosporins (11). This proposed mechanism of resistance to cephalosporins, specifically cefitoxime, has also been put forth by Neu (17). In consideration of this possible mode of resistance, it is of interest that the 7-acyl moiety ($R_2$ in Fig. 1) is thought to be a major determinant of cephalosporin potency and spectrum of activity (4). More specifically, Nakano (15) has suggested that cefitoxime’s aminothiazolyl ring at the 7-acyl position ($R_2$ moiety in Fig. 1), a structure shared by cefotaxime, cefmenoxime, and ceftriaxone, provides the affinity for penicillin-binding proteins. In light of these observations, it is possible that certain broth and blood components interact with the heterocyclic ring at the 7-acyl position of the implicated cephalosporins and alter the potency of these drugs by influencing their affinity for the penicillin-binding protein of $S.$ faecalis, thus resulting in the observed medium-associated variabilities in the activities of these cephalosporins. Although speculative, our results could support such a hypothesis, and additional studies are planned to further investigate the possibility that this mechanism may be the cause of the observed phenomenon.

These results raise two major and closely related practical questions. Which of these in vitro results is the true correct one for predicting therapeutic efficacy? Do these cephalosporins have any in vivo activity when used to treat patients with $S.$ faecalis infections? Obviously, the first question cannot be answered until the second is answered. Although there are some indications that these cephalosporins may be efficacious for treating certain enterococcal infections (26), data concerning the true efficacies of these drugs for enterococcal infections are inconclusive. If such trials are done, the $S.$ faecalis isolates should be tested for susceptibility in MH broths with and without blood to determine which results correlate with clinical efficacy. A third question that may be asked is, do other streptococci, enterococcal or nonenterococcal, show this phenomenon? In our previous study, we showed that the medium-associated discrepancies were obtained most often with $S.$ faecalis strain and also, but to a much lesser extent, with Streptococcus faecium strains (24). The medium-associated phenomenon was not observed with strains of Streptococcus bovis.

In summary, we have demonstrated that the in vitro activity (as measured by MICs) of certain second- and third-generation cephalosporins (cefuroxime, cefotaxime, cefitoxime, cefmenoxime, and ceftriaxone) on $S.$ faecalis is markedly affected by the medium in which the tests are done, whereas other cephalosporins (cephalothin, cefamandole, and cefoperazone) are affected very little. The effects of media could be correlated with the kind of broth (MH, BH1, Sceptor) used and, to a lesser degree, with the brand of MH broth used. The most marked effects, however, were associated with the addition of blood to the base media. We have postulated that the reason for this phenomenon may be interactions between medium components and the heterocyclic ring at the 7-acyl position of the affected cephalosporins that alter the cephalosporins’ activity by influencing their
affinity for penicillin-binding proteins. Correlations of this phenomenon with therapy of S. faecalis infections must await the results of clinical studies.

LITERATURE CITED