Bactericidal Activities of Chloramphenicol and Eleven Other Antibiotics Against Salmonella spp.

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The bactericidal activity of chloramphenicol against 27 strains of Salmonella typhi and 33 strains of S. enteritidis was compared with those of 11 other antibiotics. The geometric mean bactericidal concentrations of chloramphenicol against susceptible strains (36.10 and 43.13 μg/ml for S. typhi and S. enteritidis, respectively) far exceeded those of the other 11 antibiotics, with cephalothin having the next highest values (2.67 and 8.66 μg/ml) and moxalactam (0.09 and 0.28 μg/ml), cefotaxime (0.08 and 0.28 μg/ml), ceftriaxone (0.07 and 0.16 μg/ml), norfloxacin (0.06 and 0.10 μg/ml), and aztreonam (0.05 and 0.20 μg/ml) having the lowest values. The results for imipenem (0.24 and 0.81 μg/ml) and cefazidime (0.22 and 0.75 μg/ml) were lower than those noted for trimethoprim-sulfamethoxazole (1.20 and 5.56 μg/ml), cefamandole (0.62 and 3.29 μg/ml), and ampicillin (0.55 and 2.78 μg/ml). The MBC of chloramphenicol for some isolates decreased with increased incubation times such that the proportion of susceptible isolates killed by chloramphenicol at concentrations within achievable levels in blood increased from 10% after 24 h to 26% after 48 h of incubation. Although the MBCs of the other 11 antibiotics for some isolates were also lowered by prolonged incubation, all 24-h values were within achievable levels in blood. The data indicate that chloramphenicol is not uniformly bacteriostatic against S. typhi and S. enteritidis. The in vivo significance of demonstrating delayed killing by chloramphenicol is, however, uncertain.

Chloramphenicol is generally regarded as a bacteriostatic antibiotic for Salmonella spp. (19, 22). However, as with other members of the family Enterobacteriaceae (5, 12), there are occasional isolates that are killed in vitro with achievable chloramphenicol levels in blood. Recently, Asmar and Dajani (3) reported that chloramphenicol was bactericidal for 2 of 13 Salmonella isolates. To more accurately quantitate this property, we evaluated the bactericidal activity of chloramphenicol for 27 S. typhi and 33 S. enteritidis strains and compared the results with those obtained for 11 other antibiotics (ampicillin, trimethoprim-sulfamethoxazole [TMP-SMZ], cephalothin, cefamandole, moxalactam, cefotaxime, ceftriaxone, ceftazidime, imipenem, aztreonam, and norfloxacin). In addition, we studied the effect of incubation time on MIC and MBC results. Specifically, we arbitrarily used an MBC/MIC ratio of ≥10 for each antibiotic against susceptible strains as a relative measure of discrepant MBCs and MICs and hence as a relative indicator of bactericidal versus bacteriostatic activity. Values at 24 and 48 h were compared to investigate conditions that could alter this ratio and its prevalence among the Salmonella strains tested.

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MATERIALS AND METHODS

The Salmonella isolates were obtained from Frances Hickman at the Centers for Disease Control, from Bruce Kleger at the Pennsylvania State Department of Health, and from the clinical microbiology laboratory at The Children’s Hospital of Philadelphia.

Agar disk diffusion susceptibility results were determined for each isolate (13). Broth dilution susceptibility studies were carried out in duplicate in microtiter trays with Mueller-Hinton broth (14). The antibiotic concentrations used ranged from 0.05 to 100 μg/ml. Each well was inoculated to a cell density of ca. 100,000 CFU/ml. Pairs of inoculation trays were incubated at 37°C, one tray for 24 h and one tray for 48 h. The MIC was defined as the lowest concentration of antibiotic able to inhibit visible growth of the test organism. The contents of each clear well (0.1 ml) were spot-poured onto the surface of a predried Mueller-Hinton agar plate. After the spots had soaked into the agar, the plate was incubated at 37°C for 24 h. The MBC was defined as the lowest concentration of antibiotic able to kill at least 99.9% of the original inoculum (i.e., ≤10 colonies per spot).

Summary MICs and MBCs were calculated including only susceptible strains. For calculation of the geometric mean (GM) MICs and MBCs, results that were equal to or less than the lowest concentration tested were used as is, whereas results that were greater than the highest concentration tested were arbitrarily assigned a value that was one dilution higher.

RESULTS

Based on both disk diffusion and MIC results, 6 (22%) of the 27 S. typhi strains were resistant to chloramphenicol. One of these six isolates was also resistant to ampicillin, whereas one other was also resistant to TMP-SMZ. The S. enteritidis isolates were more resistant. Of the 33 isolates, 17 (52%) were resistant to ampicillin. These 17 strains included 4 (12%) that were also resistant to chloramphenicol and 9 (27%) others that were also resistant to both cephalexin and cefamandole.

In general, MICs and MBCs for S. enteritidis were higher than those for S. typhi (Tables 1 and 2). Chloramphenicol and cephalexin had the highest GM MICs for both S. typhi and S. enteritidis (for chloramphenicol, 2.60 and 2.31 μg/ml, respectively; for cephalexin, 1.76 and 3.04 μg/ml, respectively) (Table 1). On the other hand, moxalactam, cefotax-
TABLE 1. Comparative MICs of 12 antibiotics against susceptible* Salmonella isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>GM</th>
<th>Range</th>
<th>For 50% of isolates</th>
<th>For 90% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. typhi</td>
<td>S. enteritidis</td>
<td>S. typhi</td>
<td>S. enteritidis</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2.60</td>
<td>3.21</td>
<td>2.50-5.00</td>
<td>≤0.50-2.50</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>1.76</td>
<td>3.04</td>
<td>1.00-5.00</td>
<td>0.10-10.00</td>
</tr>
<tr>
<td>TMP-SMZ</td>
<td>0.71</td>
<td>0.96</td>
<td>0.50-2.50</td>
<td>0.50-5.00</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0.41</td>
<td>1.07</td>
<td>0.25-2.50</td>
<td>0.10-10.00</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.35</td>
<td>0.89</td>
<td>0.25-1.00</td>
<td>0.10-10.00</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.22</td>
<td>0.39</td>
<td>0.10-0.25</td>
<td>≤0.05-2.50</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.17</td>
<td>0.42</td>
<td>0.10-0.25</td>
<td>≤0.05-5.00</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>0.06</td>
<td>0.08</td>
<td>≤0.05-0.10</td>
<td>≤0.05-0.50</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.06</td>
<td>0.10</td>
<td>≤0.05-0.10</td>
<td>≤0.05-0.25</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.05</td>
<td>0.06</td>
<td>≤0.05-0.10</td>
<td>≤0.05-0.25</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0.05</td>
<td>0.06</td>
<td>≤0.05-0.10</td>
<td>≤0.05-0.25</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>0.05</td>
<td>0.08</td>
<td>≤0.05-0.10</td>
<td>≤0.05-0.25</td>
</tr>
</tbody>
</table>

* Excludes 6 chloramphenicol-, 1 TMP-SMZ-, and 1 ampicillin-resistant S. typhi isolate and 4 chloramphenicol-, 9 cephalothin-, 9 cefamandole-, and 17 ampicillin-resistant S. enteritidis isolates.

TABLE 2. Comparative MBCs of 12 antibiotics against susceptible* Salmonella isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>GM</th>
<th>Range</th>
<th>For 50% of isolates</th>
<th>For 90% of isolates</th>
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</tr>
<tr>
<td>Chloramphenicol</td>
<td>36.10</td>
<td>43.13</td>
<td>2.50-100.00</td>
<td>0.50-100.00</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>2.67</td>
<td>8.66</td>
<td>1.00-10.00</td>
<td>0.10-25.00</td>
</tr>
<tr>
<td>TMP-SMZ</td>
<td>1.20</td>
<td>5.56</td>
<td>≤0.50-10.00</td>
<td>0.50-50.00</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>0.62</td>
<td>3.29</td>
<td>0.25-2.50</td>
<td>0.10-25.00</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.55</td>
<td>2.78</td>
<td>0.25-2.50</td>
<td>≤0.10-25.00</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.24</td>
<td>0.81</td>
<td>≤0.05-0.50</td>
<td>≤0.05-10.00</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.22</td>
<td>0.75</td>
<td>≤0.10-1.00</td>
<td>≤0.05-10.00</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>0.09</td>
<td>0.28</td>
<td>≤0.05-0.25</td>
<td>≤0.05-2.50</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.08</td>
<td>0.28</td>
<td>≤0.05-0.50</td>
<td>≤0.05-1.00</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.07</td>
<td>0.16</td>
<td>≤0.05-0.25</td>
<td>≤0.05-1.00</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0.06</td>
<td>0.10</td>
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SMZ). On the other hand, all the other antibiotics had discrepant MBCs for the *S. enteritidis* strains, with rates varying from a high of 52% for TMP-SMZ to a low of 6% for norfloxacin. Furthermore, whereas inordinately high MBCs for more than one antibiotic besides chloramphenicol occurred with only 5% of the *S. typhi* strains, this property was noted with 41% of the *S. enteritidis* strains. In no case, however, did the MBCs of the other 11 antibiotics for any isolate exceed achievable levels in blood.

After 48 h of incubation, none of the other 11 antibiotics had an MBC/MIC ratio of ≥10 against any of the *Salmonella* strains (Table 3). The change in MBCs and MICs observed for TMP-SMZ against the *S. enteritidis* strains initially with an MBC/MIC ratio of ≥10 are depicted in Fig. 2. As was the case for chloramphenicol, the change in the MBC/MIC ratio was due to a change in MBCs. The 24- and 48-h GM MBCs were 17.42 and 1.25 μg/ml, respectively; the corresponding GM MICs were 0.78 and 1.07 μg/ml, respectively.

![FIG. 1. Change in MBCs and MICs (for susceptible strains with an MBC/MIC ratio of ≥10 at 24 h of incubation) of chloramphenicol for *Salmonella* spp. with incubation time. ——, GM values.](http://aac.asm.org/)

**DISCUSSION**

Our data document that *S. typhi* and *S. enteritidis* are exquisitely susceptible in vitro to a number of newly licensed and soon-to-be-licensed antibiotics. Although comparisons of antibiotic activities frequently include resistant organisms, we excluded such isolates to prevent an artificial increase in the difference between antibiotics to which resistance has occurred and those to which resistance has not yet been observed.

These findings are important in light of chloramphenicol and ampicillin (and amoxicillin) resistance. Unfortunately, in vitro results for *Salmonella* spp. may not correlate with clinical response (1, 7, 24). Although cefazolin may be effective (24), the only alternative antibiotic to date that has been relatively well studied and shown to be efficacious has been TMP-SMZ (4); however, resistance to this antibiotic has been reported (20, 21). Fortunately, favorable results for other, newer antibiotics are accumulating (2, 5, 10).

Our results also document that, in general, chloramphenicol is bacteriostatic against *Salmonella* strains in vitro. However, the percentage of isolates that was killed by chloramphenicol at concentrations <10 times the MIC was influenced by the time of incubation, with an increase from 10% at 24 h to 26% after 48 h incubation. Although the MBC/MIC ratio of ≥10 was arbitrarily chosen, it did correlate with MBCs that would not be attainable using standard chloramphenicol dosages. Of interest is the finding that the MBC/MIC ratios of ≥10 obtained after 24 h of incubation for the other 11 antibiotics uniformly decreased to low values after 48 h of incubation. However, the 24-h MBCs were still within achievable levels in blood.

The bacteriostatic nature of chloramphenicol may in some way be involved with the therapeutic problems associated with chloramphenicol therapy of *S. typhi*, namely, relapse and a carrier state (4, 27). In addition, the bacteriostatic activity of chloramphenicol should be considered when treating other serious *Salmonella* infections, especially meningitis (5, 15, 18, 19). Furthermore, there are some data suggesting that combining chloramphenicol with ampicillin to treat serious *Salmonella* infections may be useful only if
chloramphenicol is bactericidal for the particular organism (3). Otherwise, antagonism between the two antibiotics may occur. This finding may have clinical implications regarding routine combination therapy (3, 6, 8, 18).

Our observed laboratory-dependent changes in MBCs for the antibiotics studied are not unique. Alterations in MBCs have been documented for other antibiotics against other organisms after increased incubation times (23, 26) as well as after changes in broth (17), temperature (9), pH (25), and inoculum density (11, 16). Although the changes in MBCs noted after prolonged incubation may simply be in vitro phenomena, it is intriguing to speculate that similar changes do occur in the blood, cerebrospinal fluid, and tissues, assuming that it may take various times for susceptible organisms to be killed once they are exposed to a given antimicrobial agent. The true clinical importance of this laboratory phenomenon is, however, not known.

ACKNOWLEDGMENT

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LITERATURE CITED