Susceptibility of 40 Lactobacilli to Six Antimicrobial Agents with Broad Gram-Positive Anaerobic Spectra

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The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of 40 lactobacillus strains were determined against six antibiotics with broad anaerobic spectra. Penicillin, ampicillin, clindamycin, and cephalothin were the most active inhibitory agents, with 95 to 100% of the strains inhibited at clinically achievable serum levels. However, despite the inhibitory efficacy of these four agents, only 5 to 22% of the isolates were killed at achievable concentrations. MBC:MIC ratios were high, ranging from 30:1 for cephalothin to 266:1 for ampicillin. Cefoxitin and metronidazole were generally ineffective against lactobacilli, with 87.5 to 100% of strains having unachievable MICs and/or MBCs. These findings may partially explain the clinical observations noting the inability to eradicate endocarditic lactobacillemias despite readily achievable MICs.

Lactobacilli are being increasingly recognized as important pathogens in human infections (1, 3, 17, 20). An intriguing phenomenon noted in many previously cited cases of lactobacillemia was the therapeutic difficulty in eradicating this organism from deep-seated infected foci, especially the endocardium, despite seemingly appropriate treatment regimens and serum antibiotic concentrations exceeding the minimal inhibitory concentrations (MICs).

To investigate this problem of apparent discrepancy between in vitro susceptibility of lactobacilli and poor in vivo therapeutic responses, the MICs and minimal bactericidal concentrations (MBCs) of six antibiotics with broad gram-positive anaerobic spectra were determined by a modified broth dilution technique for 40 clinical isolates.

MATERIALS AND METHODS

Isolation of the lactobacilli. All 40 strains of lactobacilli were clinical isolates from a variety of body sites, including blood, genital tract, oropharynx, ascitic fluid, lymph node, and brain tissue. Ten were obtained from the clinical laboratory of Harbor General Hospital, whereas the remaining 30 organisms were kindly provided by the following investigators: Elizabeth P. Cato, Blacksburg, Va.; Robert E. Weaver, Atlanta, Ga.; Jack P. London, Bethesda, Md.; and M. Elizabeth Sharpe, Reading, England.

The lactobacilli were identified by typical appearance on Gram stain and by biochemical reactions according to Bergey’s criteria (14). Identification of species was according to classifications of Holdeman and Moore (12). The following strains of lactobacilli were used in this study: Lactobacillus casei (24 strains), L. plantarum (5 strains), L. acidophilus and L. minutis (3 strains each), L. fermentans and L. brevis (2 strains each), and L. leichmani (1 strain). All of the L. plantarum and L. casei and two of the three L. minutis isolates were facultative anaerobes; the remainder were strict anaerobes.

Organisms were maintained in chopped-meat glucose (CMG) broth (Scott Laboratories, Fiskeville, R.I.) and were transferred monthly into fresh media until the susceptibility testing was performed. In the week before testing, one additional transfer was done.

Preparation of media and antibiotics. Preehanced Mueller-Hinton (MH) broth (Baltimore Biological Laboratory, Cockeysville, Md.) was freshly prepared for use in this study. In preliminary pilot studies in our laboratory, we compared the overnight turbidometric and quantitative dilution plate growth of 10 of the isolates (five facultatives and five of the strict anaerobes) in prereduced MH and Schaedler broth; the nephelometer and colony count data were comparable, and the MIC end points were more sharply definable in prereduced MH broth. In addition, the MBCs were virtually identical in the two media. We thus chose to use prereduced MH broth in this study.

Serial twofold dilutions were made of all antibiotics and added to the MH broth under strictly anaerobic conditions. After addition of the lactobacillus inocula, the final range of drug concentration was 0.312 to 320 μg/ml for penicillin, ampicillin, cephalothin, clindamycin (USP Standards, Rockville, Md.), metronidazole (Searle, Chicago, Ill.), and cefoxitin (Merck Sharpe, & Dohme, Rahway, N.J.).

Susceptibility testing. MICs and MBCs of the six antibiotics to the 40 strains of lactobacilli were determined by a modified broth dilution technique (18). For
LACTOBACILLUS ANTIMICROBIAL SUSCEPTIBILITIES

Table 1. MIC and MBC for various fractions of 40 lactobacilli to six anaerobic agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Generally achievable serum concn (µg/ml)</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25% 50% 75% 95% 25% 50% 75% 95%</td>
<td></td>
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</tr>
<tr>
<td>Penicillin</td>
<td>10 31 42 0.58 0.99 10 &gt;320 &gt;320 &gt;320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>5 0.24 0.49 0.94 4.37 14 45 114 &gt;320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20 0.39 0.82 2.2 8.3 165 216 267 320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>20 3.0 6.75 9.7 20 40 &gt;320 &gt;320 &gt;320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>20 53 227 &gt;320 &gt;320 &gt;320 &gt;320 &gt;320 &gt;320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metronidazole</td>
<td>12.5 140 &gt;320 &gt;320 &gt;320 &gt;320 &gt;320 &gt;320 &gt;320</td>
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</tr>
</tbody>
</table>

* MIC of 95% of the 40 isolates.
ance to both the bacteriostatic and bactericidal effects of cefoxitin and metronidazole. These latter findings are similar to those seen with other anaerobic, gram-positive bacilli, such as certain nonperfringens Clostridia and Actinomyces, which also demonstrate relative resistance to metronidazole or cefoxitin (6,11,13,19). Metronidazole selectively inhibits anaerobes, probably by serving as a specific electron acceptor for reduced ferredoxin. This action interferes with electron transfer in the phosphoroclastic reaction found only in anaerobic and microaerophilic organisms (7, 8). The relative resistance of the lactobacilli and other gram-positive, anaerobic bacilli to this specific action of metronidazole suggests that these organisms either bypass the phosphoroclastic reaction with other metabolic pathways or block the electron transfer inhibitions of metronidazole (6).

The MBC:MIC ratios were high, ranging from 30:1 for cephalothin to 266:1 for ampicillin. Moreover, for the six agents tested, the MBC exceeded its corresponding MIC by $\geq 100$-fold in 47% of paired MBC-MIC determinations. These observations are suggestive that the lactobacilli demonstrate antibiotic "tolerance" as delineated by Sabath and others (15).

Clinically, lactobacillemias associated with deep-seated infectious foci, particularly the endocardium, have been relatively refractory to high-dose intravenous therapy with the penicillins and cephalothin, despite readily achievable MICs to these agents. A significant proportion of such cases of lactobacillus endocarditis have been resistant to treatment with intravenous penicillin in doses of $20 \times 10^6$ to $30 \times 10^6$ U daily (1, 3, 20). The widely disparate MBC-MIC results observed in the present study may partially explain the discrepancy between achievable MICs in vitro and the suboptimal therapeutic responses in vivo of endocarditic lactobacillemias. Of note, preliminary synergistic studies suggest the efficacy of penicillins in combination with aminoglycosides against lactobacilli at clinically achievable levels for both agents (3).

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