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. Elisabeth 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. minutus (3 strains each), L. fermentans and L. brevis (2 strains each), and L. leichmanii (1 strain). All of the L. plantarum and L. casei and two of the three L. minutus 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. Preserved 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
each isolate, 0.1-ml aliquots of the CMG broth maintenance culture were added under anaerobic conditions to prereduced MH broth. This was incubated for 24 h at 37°C and adjusted to a McFarland no. 1 nephelometer standard (2) previously determined to approximate 2 × 10^9 colony-forming units per ml. The nephelometer approximations were routinely confirmed by formal dilution plate colony counts for each determination. Inocula of approximately 2 × 10^9 colony-forming units were added anaerobically to each antibiotic-containing tube. After the tubes were incubated for 24 h at 37°C, the MIC was determined as the least concentration of the antibiotic in which there was no visible growth. MBCs were determined by subculturing 0.1-ml aliquots from each tube onto blood agar plates. All plates were incubated at 37°C for 48 h in anaerobic incubation jars after air had been evacuated and replaced with a gas mixture containing 80% N\textsubscript{2}, 10% H\textsubscript{2}, and 10% CO\textsubscript{2} (Gas-Pak-100, Baltimore Biological Laboratory). The MBC was defined as the lowest antibiotic concentration yielding five or less visible colonies on agar. An L. plantarum with known MICs and MBCs to the antibiotics tested was included in each determination for reproducibility.

**Statistical analysis.** Geometric mean MICs and MBCs were compared by the Student's t test.

**RESULTS**

The MICs and MBCs for various fractions of the 40 test strains are summarized in Table 1. The geometric mean MICs and MBCs were also calculated against the six agents used. By employing the generally attainable serum levels of these antibiotics (4, 5, 9, 10, 16) as susceptibility “break points,” the following data were observed.

**Penicillin.** The geometric mean MIC for penicillin was 0.48 μg/ml. However, the geometric mean MBC was >100 μg/ml. Thus, 100% of strains were inhibited by penicillin, but only 22% were killed at generally achievable serum levels. Similarly, for ampicillin, the geometric mean MIC was 1.32 μg/ml, whereas the geometric mean MBC was >350 μg/ml. Thus, 97% of strains were inhibited by ampicillin, but only 5% were killed by this agent.

**Cephalosporins.** The geometric mean MIC for cephalothin was 6.6 μg/ml, with 95% of strains inhibited by this agent; the geometric mean MBC was >198 μg/ml, with only 15% of strains killed by cephalothin at achievable levels. Cefoxitin was relatively ineffective against the lactobacilli, with geometric mean MIC and MBC of 106 and >423 μg/ml, respectively. Thus, only 12.5% of strains were inhibited by this agent, and none was killed.

**Clindamycin.** The geometric mean MIC was 0.73 μg/ml, and the geometric mean MBC was 45 μg/ml. Thus, 98% of strains were inhibited by clindamycin; however, this agent was bactericidal for only 17.5% of the isolates.

**Metronidazole.** Metronidazole was totally ineffective against the lactobacilli, with a geometric mean MIC and MBC of >503 and >563 μg/ml, respectively.

**MBC:MIC ratios.** The geometric mean MBC:MIC ratios for the four most active antibiotics (penicillin, ampicillin, clindamycin, and cephalothin) against these 40 strains of lactobacilli were: (i) penicillin, 210:1; (ii) ampicillin, 266:1; (iii) clindamycin, 62:1, and (iv) cephalothin, 30:1.

**Statistical analyses.** Although the number of strict anaerobes was relatively small (9/40), there were no statistical differences in MICs or MBCs for the six antimicrobials when the facultative isolates were contrasted with the strict anaerobes (P > 0.10).

**DISCUSSION**

This study confirmed our preliminary data (3) concerning widely disparate MICs and MBCs of the lactobacilli to agents with broad gram-positive anaerobic spectra. Among the four most active inhibitory agents (i.e., penicillin, ampicillin, clindamycin, and cephalothin), 95% or more of the 40 strains were inhibited by each antibiotic. However, the most active bacteriostatic agent, penicillin, was bactericidal for <25% of strains. Similarly, ampicillin, clindamycin, and cephalothin each were bactericidal to less than 20% of strains at clinically achievable levels. The lactobacilli demonstrated high-magnitude resist-

<table>
<thead>
<tr>
<th>Agent</th>
<th>Generally achievable serum concn (μg/ml)</th>
<th>MIC (μg/ml)</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>95%*</th>
<th>MBC (μg/ml)</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>10</td>
<td>0.31</td>
<td>0.42</td>
<td>0.58</td>
<td>0.99</td>
<td>10</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>5</td>
<td>0.24</td>
<td>0.49</td>
<td>0.94</td>
<td>4.37</td>
<td>14</td>
<td>45</td>
<td>114</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20</td>
<td>0.39</td>
<td>0.82</td>
<td>2.2</td>
<td>8.3</td>
<td>165</td>
<td>216</td>
<td>267</td>
<td>320</td>
<td>320</td>
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</tr>
<tr>
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<td>20</td>
<td>3.0</td>
<td>6.75</td>
<td>9.7</td>
<td>20</td>
<td>40</td>
<td>&gt;320</td>
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</tr>
<tr>
<td>Cefoxitin</td>
<td>20</td>
<td>53</td>
<td>227</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
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<td>Metronidazole</td>
<td>12.5</td>
<td>140</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
<td>&gt;320</td>
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* 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 ferrodoxin. 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 >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, lactobacillales 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 × 10⁶ to 30 × 10⁶ U daily (1, 3, 20). The widely disparate MBC-MIC results observed in this study may partially explain the discrepancy between achievable MICs in vitro and the suboptimal therapeutic responses in vivo of endocarditic lactobacillales. 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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LITERATURE CITED