In Vitro Susceptibility of Escherichia coli O157 to Several Antimicrobial Agents

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We evaluated the antimicrobial susceptibility of six strains of Escherichia coli O157 (E. coli O157) isolated from patients in Yamaguchi Prefecture between June and July, 1996. Seven antimicrobial agents that were expected to retain a high concentration in the intestine were selected. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of ciprofloxacin, polymyxin B, cefoperazone, and kanamycin for each strain were ≤6.25 μg/mL. However, the MIC of fosfomycin was 3.13–100 μg/mL, and its MBC was ≥100 μg/mL. The MIC of ampicillin and tetracycline was >100 μg/mL in some strains. In a time-kill study of E. coli O157 at a drug concentration of 12.5 μg/mL, after 104 colony forming units/mL of E. coli O157 were eradicated within 10 min by ciprofloxacin, within 30 min by polymyxin B, within 4 h by cefoperazone, and within 16 h by kanamycin. These results suggest that the new quinolones with a poor absorption rate in the intestine (such as ciprofloxacin and norfloxacin) are effective against E. coli O157. When oral administration is impossible, bile excreting cephal antibiotics (such as cefoperazone, ceftriaxone, and cefotetan) may be useful.

Key words Escherichia coli O157; enterohemorrhagic E. coli; antimicrobial susceptibility; ciprofloxacin; polymyxin B

Food poisoning caused by Escherichia coli O157 (E. coli O157) occurred in Japan in May 1996. Since few studies have examined the susceptibility of E. coli to antimicrobial agents however, appropriate medical therapy is not always possible. We evaluated the susceptibility of this microorganism to 7 antimicrobial agents.

MATERIALS AND METHODS

Organisms Six strains of E. coli O157 isolated from 6 patients living in 5 municipalities in Yamaguchi Prefecture (3 males and 3 females aged 8–73 years) between June 19 and July 22, 1996 were used. Of the 6 strains, 5 were strains producing both verotoxin I and II, and the other was a verotoxin II producing strain.

Agents The bulk powders of the following 7 antimicrobial agents were used: ciprofloxacin (Bayer Yakuhin, Ltd.), polymyxin B sulfate (Pfizer Pharmaceuticals Inc.), kanamycin sulfate, fosfomycin Na, ampicillin Na (Meiji Seika Kaisha, Ltd.), cefoperazone Na (Toyama Chemical Co., Ltd.), and tetracycline HCl (Lederle Japan, Ltd.).

Sensitivity Testing The minimum inhibitory concentration (MIC) was determined by the two-fold broth dilution method. Bacterial solution incubated in Mueller–Hinton broth (Difco) at 35°C for 18 h was diluted to a concentration of about 10⁶ colony forming units (CFU)/ml with 5 ml of Mueller–Hinton broth containing each drug in the two-fold dilution series. After incubation at 35°C for 18 h, MIC was determined.

The minimum bactericidal concentration (MBC) was determined by the following method. From the culture showing no turbidity in the above Mueller–Hinton broth, 10 μl was inoculated into 10 ml of Mueller–Hinton broth and cultured at 35°C for 18 h.

Time-Kill Study This experiment was performed in one strain of E. coli O157. Bacterial solution incubated in Mueller–Hinton broth at 35°C for 18 h was diluted with 5 ml of Mueller–Hinton broth to a concentration of about 10⁶ CFU/ml. After incubation at 35°C for 2 h, each antimicrobial agent was added, and incubation was continued at 35°C. After the cultivation, 0.5 ml of the culture was poured into 10 ml of physiological saline, and passed through a 0.22-μm membrane filter (diameter, 6 cm; Becton Dickinson & Co.). In addition, to prevent antibiotic carry-over, 100 ml of physiological saline was passed through this membrane filter twice. The filter was incubated in Trypto-soy agar (Eiken Chemical Co., Ltd.) at 35°C for 18 h, and the colony formed on the membrane filter was counted. A viable count >100 CFU was defined as +++, 10–100 CFU as ++, and 1–9 CFU as +.

RESULTS

MIC and MBC Table 1 shows the MIC and MBC of the 7 agents against the 6 strains of E. coli O157. Both MIC and MBC of ciprofloxacin, polymyxin B, cefoperazone, and kanamycin were low for each strain; in particular, the MIC and MBC of ciprofloxacin and polymyxin B were 0.39 μg/mL or less. On the other hand, the MIC of fosfomycin was 100 μg/mL for one strain, and its MBC was 100 μg/mL or more for all strains. Ampicillin

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Seven Agents against Six Strains of E. coli O157

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (μg/mL)</th>
<th>MBC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.2</td>
<td>≤0.2</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>≤0.2</td>
<td>≤0.2–0.39</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>≤0.2–1.56</td>
<td>≤0.2–1.56</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>3.13–6.25</td>
<td>3.13–6.25</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>3.13–100</td>
<td>≥100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3.13–&gt;100</td>
<td>6.25–&gt;100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.78–&gt;100</td>
<td>≥100</td>
</tr>
</tbody>
</table>

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Table 2. Effect of Four Agents at a Concentration of 12.5 μg/ml on E. coli O157 at 35 °C

<table>
<thead>
<tr>
<th>Agent</th>
<th>Growth after exposure for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>−</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>+</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>+++</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>+++</td>
</tr>
</tbody>
</table>

0h: 2.6 x 10⁴ colony forming units/ml. ++++, >100 CFU; ++, 10–100 CFU; +, 1–9 CFU; −, no growth.

and tetracycline showed a MIC of more than 100 μg/ml for some strains.

Table 2 shows the time required for killing of an E. coli O157 strain by 4 of the antimicrobial agents. The bacteria were eradicated within 10 min by ciprofloxacin, within 30 min by polymyxin B, within 4 h by cefoperazone, and within 16 h by kanamycin.

DISCUSSION

We selected 7 antimicrobial agents that are expected to retain a high concentration in the intestine and evaluated their in vitro effectiveness against E. coli O157. Among these agents, ciprofloxacin showed a low MIC and a low MBC as well as rapid bactericidal effects. Previous studies have noted a low resistant rate (2.0%) of E. coli to ciprofloxacin and the usefulness of this agent against shigellosis. Our results together with these previous findings suggest the benefit of ciprofloxacin for treating E. coli O157 infection. E. coli has also been reported to show a low resistant rate (1.8%) to norfloxacin, a new quinolone with a low absorption rate in the digestive tract. This agent can also be used for E. coli O157 infection.

Polymyxin B also showed a low MIC and a low MBC and rapid bactericidal effects. Polymyxin B has been reported to be highly active against E. coli. However, this agent has been used to release verotoxin from E. coli O157 in an in vitro study. Therefore, further studies are needed to determine whether polymyxin B is an appropriate agent for the treatment of E. coli O157.

Cefoperazone had less rapid bactericidal effects than ciprofloxacin or polymyxin B on E. coli O157 but showed relatively low MIC and MBC. The resistance rate of E. coli to this agent is not high (3.3%). Therefore, when oral administration is impossible in patients with E. coli O157 infection, cefoperazone can be a drug of choice. Ceftriaxone, cefotetan, and ceftobiprole, which are bile excreting type cepham antibiotics like cefoperazone, can also be selected.

Though kanamycin showed relatively low MIC and MBC in this study using 6 bacterial strains, its rapid bactericidal effects were the lowest among the 4 agents examined. Further, the resistance rate of E. coli to kanamycin has been reported to be more than 10% (9), therefore, this agent cannot be recommended for E. coli O157 infection. Fosfomycin, ampicillin, and tetracycline showed an MIC or MBC of 100 μg/ml or more for some strains even in this experiment where only 6 strains were used. Therefore, these agents also should not be used for E. coli O157 infection.

The results of this study together with drug kinetics and the resistance rate of E. coli to each agent show the effectiveness of the new quinolones ciprofloxacin and norfloxacin against E. coli O157 infection.

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REFERENCES