Comparative In Vitro Activities of Amoxicillin-Clavulanic Acid, Cefuroxime, Cephalexin, and Cephalothin against Trimethoprim-Resistant Escherichia coli Isolated from Stools of Children Attending Day-Care Centers

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A high prevalence of fecal colonization with trimethoprim-resistant Escherichia coli was found in diapered children attending day-care centers in Houston, Tex. In the present study, 100 isolates of E. coli resistant to multiple antibiotics, including trimethoprim (100%), sulfisoxazole (100%), streptomycin (94%), and ampicillin (87%), were obtained over a 5-month period from stool samples of diapered children attending four day-care centers and tested for their susceptibilities to amoxicillin-clavulanic acid, cefuroxime, cephalexin, and cefalothin. The MICs for 50 and 90% of strains tested were 16 and 32 μg/ml, respectively, for amoxicillin-clavulanic acid, 4 and 16 μg/ml, respectively, for cefuroxime, 4 and 64 μg/ml, respectively, for cephalexin, and 32 and >64 μg/ml, respectively, for cefalothin. Although all three oral beta-lactams tested were generally active at concentrations likely to be achieved in urine, cefuroxime and cephalexin were more potent and are thus more likely to be inhibitory at the concentrations needed for systemic infections.

Antibiotic resistance is the single most important factor that limits the long-term efficacy of antimicrobial agents. Certain settings, such as intensive-care units, animal feedlots, and developing countries, have traditionally been associated with the emergence of antibiotic-resistant bacteria (5). Recently, health care providers have focused attention on the relationship between day-care centers (DCCs) and antibiotic-resistant bacteria (3). The DCC environment is conducive to the spread of antibiotic-resistant bacteria, especially among diapered children, for several potential reasons. First, the close contact of these children facilitates transmission; second, diapered children have not learned personal hygiene practices and defecate indiscriminately; and third, antibiotics are widely used in this population (9).

In our initial study of 79 children under 2 years of age in DCCs in 1986, we found colonization of stool with trimethoprim- and ampicillin-resistant gram-negative bacteria in 28 and 65% of the children, respectively; over 80% of the isolates were Escherichia coli (11). Most of the trimethoprim-resistant E. coli were also resistant to sulfisoxazole, ampicillin, and streptomycin. These results were significant because they represented the first documentation in the United States of high rates of colonization with trimethoprim-resistant (TMP) E. coli in stools of individuals who were not hospitalized and who were not receiving chronic therapy with trimethoprim-sulfamethoxazole. A later study found that 30% of 203 children in 12 DCCs were colonized with TMP E. coli, with rates in individual DCCs ranging from 0 to 59% (10); these organisms were also resistant to sulfisoxazole (100%), streptomycin (96%), and ampicillin (94%).

The finding that a remarkably high percentage of children in some DCCs harbor TMP E. coli suggests that DCCs are a potential source of bacteria resistant to multiple antibiotics. The present study was undertaken to determine the in vitro activities of several broad-spectrum antibiotics, including cefuroxime, cephalexin, cefalothin, and amoxicillin-clavulanic acid, against 100 isolates of multiresistant E. coli.

(MATERIALS AND METHODS

Study population. The 91 children from whom E. coli isolates were obtained were part of a 5-month prospective study of the acquisition and persistence of TMP E. coli among diapered children less than 24 months of age attending four DCCs in Houston, Tex. (Fornasini et al., 29th ICAAC). As in our previous studies (10, 11), written informed consent from each DCC director and permission from the parents of the enrolled children were obtained. This study was approved by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston.

Isolation of multiresistant bacteria. Research nurses visited the DCCs and collected stool samples every 2 to 4 weeks. Two methods were used to detect TMP organisms: a semiquantitative dilution method described previously (11) and a plate detection method in which approximately 1.5 mg of stool was streaked onto MacConkey agar with trimethoprim (50 μg/ml). Organisms which grew on the trimethoprim-containing medium were considered TMP if they also grew when they were restreaked onto Mueller-Hinton agar containing 50 μg of trimethoprim per ml.

Plasmid profile and identification. Five E. coli-like colonies, including representatives of all colonial morphologies, were selected from the trimethoprim-containing agar and analyzed for their plasmid content by the lysis method of Kado and Liu (4) and electrophoresis in 0.7% agarose gels. From each specimen, every isolate which had a distinct total plasmid profile was selected and identified by standard biochemical tests.

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Susceptibility testing. Isolates of *E. coli* resistant to trimethoprim (as defined by growth on Mueller-Hinton agar with trimethoprim at 50 μg/ml) were chosen for susceptibility testing based on the observation that the isolate had a different plasmid pattern from those of other isolates being tested, that the isolates were from different children, or both. Isolates from the same child were tested only if the plasmid pattern indicated that they clearly represented different strains (B. E. Murray and S. L. Hodel-Christian, in V. Lorian, ed., *Antibiotics in Laboratory Medicine*, in press). Isolates were tested for susceptibility to ampicillin, cefuroxime, cephalexin, chloramphenicol, gentamicin, streptomycin, sulfisoxazole, tetracycline, trimethoprim, and trimethoprim-sulfamethoxazole by the disk diffusion method with Mueller-Hinton agar (11); disks were obtained from BBL.

MICs of amoxicillin-clavulanic acid, cefuroxime, cephalexin, and cephalothin (obtained from their respective manufacturers) were determined by agar dilution by using serial twofold dilutions in Mueller-Hinton agar according to the recommendations of the National Committee for Clinical Laboratory Standards (6). An inoculum replicating device was used to give a final inoculum of 10⁶ CFU per spot. MICs of all antimicrobial agents were recorded as the lowest concentration that inhibited visible growth. Antimicrobial concentrations that inhibited 50 and 90% of the isolates and the percentage of resistant isolates were calculated in accordance with National Committee for Clinical Laboratory Standards interpretive breakpoints for susceptible, moderately susceptible, and resistant (6). These values are ≤8/4, 16/8, and ≥32/16 μg/ml for amoxicillin-clavulanate; ≤8, 16, and ≥32 μg/ml for cefuroxime sodium; ≤4, 8 to 16, and ≥32 μg/ml for cefuroxime axetil; and ≤8, 16, and ≥32 μg/ml for cephalexin, respectively. Cephalothin is considered the class compound for testing of cephalexin. For the oral agents, the moderately susceptible classification is probably appropriate only for urinary tract infections based on concentrations achieved in urine.

RESULTS

Based on analysis of over 900 isolates of TMP* E. coli* collected during the epidemiological study, we chose 100 isolates for further study. Forty isolates were selected because we were able to identify 40 clearly different plasmid patterns (examples of which are shown in Fig. 1). An additional 24 isolates had plasmid patterns that differed from one to two plasmid bands; these isolates may be different strains or could represent variants of the 40 strains that gained or lost all or part of a plasmid(s) (Murray and Hodel-Christian, in press). Several strains had no visible plasmids. Thirty-three isolates had the same plasmid pattern as that of another strain tested but were obtained from a different child at a different point in time. Although this means that some strains were represented more than once, an effort to evaluate only distinct strains is rarely, if ever, made in susceptibility studies.

By disk diffusion testing, all 100 TMP* E. coli* isolates were resistant to sulfisoxazole and trimethoprim-sulfamethoxazole, 94% were resistant to streptomycin, 87% were resistant and 7% were moderately susceptible to ampicillin, and 38% were resistant to chloramphenicol and tetracycline; only one isolate was resistant to gentamicin. All isolates were resistant to at least three of the individual agents listed above and are thus referred to as multiresistant; most isolates were resistant to four of these agents. Disk susceptibility test results were also determined for cefuroxime and cephalexin and showed 7% and 61% resistance, respectively (data not shown).

The comparative in vitro activities of amoxicillin-clavulanic acid, cefuroxime, cephalexin, and cephalothin against the 100 isolates of multiresistant *E. coli* are summarized in Table 1. By using the National Committee for Clinical Laboratory Standards standard interpretive breakpoints of resistance as ≥32 μg/ml, 7% of strains were resistant to cefuroxime, 19% were resistant to amoxicillin-clavulanic acid, and 77% were resistant to cephalexin; 12% of isolates were resistant to ≥32 μg of cephalexin per ml. Cumulative susceptibilities to cefuroxime at 4, 8, and 16 μg/ml were 50, 83, and 93%, respectively; the highest MIC was 64 μg/ml (four isolates). For cephalexin, cumulative susceptibilities at these concentrations were 77, 88, and 88%, respectively, while for five isolates MICs were 64 μg/ml and for seven isolates MICs were >64 μg/ml. For amoxicillin-clavulanic acid, 9, 36, and 81% were inhibited at 4, 8, and 16 μg/ml, respectively.

![FIG. 1. Agarose gel showing the total plasmid content of 15 isolates. Plasmid patterns that appeared to be similar, e.g., lanes c and g or lanes e and k, were run side by side in the same gel before interpretation.](http://aac.asm.org/)

**TABLE 1. Comparative in vitro activities of antimicrobial agents against 100 isolates of multiresistant *E. coli***

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml)*</th>
<th>50%</th>
<th>90%</th>
<th>≤2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>&gt;64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefuroxime</td>
<td>4</td>
<td>16</td>
<td></td>
<td>10</td>
<td>40</td>
<td>33</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>4</td>
<td>64</td>
<td></td>
<td>13</td>
<td>64</td>
<td>11</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>16</td>
<td>32</td>
<td></td>
<td>5</td>
<td>4</td>
<td>27</td>
<td>45</td>
<td>17</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>32</td>
<td>&gt;64</td>
<td></td>
<td>4</td>
<td>8</td>
<td>11</td>
<td>33</td>
<td>21</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

*50% and 90%, MICs for 50 and 90% of isolates tested, respectively.*
respectively; for two isolates MICs were >64 μg/ml. Cephalothin was much less active (Table 1).

DISCUSSION

The possibility of the spread of resistant bacteria from DCCs into families and then into the general community could represent a potential public health problem. A Swedish study showed that TMP E. coli can be transmitted among family members (12). Family members of outpatients with urinary tract infections caused by TMP E. coli (study group) submitted stool samples, as did family members of outpatients with urinary tract infections caused by TMP E. coli (control group). Isolates of TMP E. coli were obtained from more than 30% (16 of 51) of the family members in the study group, whereas these isolates were found in 2% (1 of 46) of the control group, indicating that transmission within families is not unusual. It is noteworthy that TMP E. coli could be recovered in the feces of family members in the study group several weeks after the original urinary culture, suggesting that the presence of TMP E. coli is not a transient phenomenon. We have also found TMP E. coli more often in family members of children colonized with TMP strains and have shown, using plasmid profile analysis, the presence of the same plasmid in stools of children and family members (Fornasini et al., 29th ICACAC).

Since the fecal flora is the usual source of urinary tract pathogens, the presence of TMP E. coli in DCCs is likely a harbinger of infections caused by these organisms in children in DCCs, their siblings, or family members. In an effort to identify other potentially useful agents, we investigated the susceptibility of multiresistant E. coli obtained from stool samples of children attending DCCs to orally administered beta-lactams and compared this activity with that of cephalothin; all organisms were resistant to trimethoprim-sulfamethoxazole, and 87% were resistant to ampicillin by disk diffusion. Cephalothin was the least active compound, with 77% of isolates being resistant to ≥32 μg/ml. Cefuroxime and cephalexin were the most active agents tested; for only 7 and 12 strains, respectively, were the MICs ≥32 μg/ml. For no strain was the MIC of cefuroxime >64 μg/ml, while for seven strains MICs were >64 μg of cephalexin per ml. MICs of amoxicillin-clavulanic acid (expressed as the concentration of amoxicillin) were generally one to two dilutions higher than those of cefuroxime and cephalexin; for 17 strains MICs were 32 μg/ml and for 2 strains MICs were >64 μg/ml. These results are in general agreement with those of previous studies in which the antimicrobial activities of various of these agents have been compared (1, 2, 7, 8, 13). Considering the National Committee for Clinical Laboratory Standards breakpoints for susceptibility to cefuroxime axetil (4 μg/ml), cefuroxime sodium (8 μg/ml), and amoxicillin-clavulanic acid (8/4 μg/ml), 50% of strains would be classified as susceptible to orally administered cefuroxime, 83% would be classified as susceptible to the parenteral form, and 36% would be classified as susceptible to amoxicillin-clavulanic acid. Because peak concentrations of active drug in serum following oral consumption of cefuroxime axetil and amoxicillin-clavulanate are comparable (4.1 to 7.0 versus 4.4 to 7.6 μg/ml, respectively) following usual dosing, the clinical significance of the difference in the breakpoints for susceptibility for these two compounds is unknown.

In conclusion, cefuroxime, cephalexin, and amoxicillin-clavulanic acid were generally active at concentrations that are likely to be achieved in urine against trimethoprim-sulfamethoxazole-resistant E. coli, including those that are ampicillin resistant, although cefuroxime and cephalexin were more potent. Since the fluoroquinolones, which are also highly active against E. coli, are not approved for use in children, these beta-lactams may be valuable alternatives for the treatment of infections caused by multiresistant E. coli, particularly in children attending DCCs. However, only clinical experience can validate the actual usefulness of these agents in the DCC setting.

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LITERATURE CITED


