Antimicrobial Susceptibility Testing of 59 Strains of Campylobacter fetus subsp. fetus

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The susceptibilities of 59 Campylobacter fetus subsp. fetus isolates to eight antibiotics were studied by the agar dilution, E-test, and disk diffusion methods. None of the isolates were β-lactamase producers. All were susceptible to ampicillin, gentamicin, imipenem, and meropenem as determined by the three methods, with MICs at which 90% of the isolates are inhibited (MIC90s) (determined by agar dilution) of 2, 1, ≤0.06, and 0.12 μg/ml, respectively. Twenty-seven percent of the isolates were resistant to tetracycline, with complete agreement between the agar dilution and disk diffusion results. The MIC90s determined by agar dilution were 2 μg/ml for erythromycin, 1 μg/ml for ciprofloxacin, and 8 μg/ml for ceftoxime.

Campylobacter fetus subsp. fetus is a rare human pathogen that causes extraintestinal infections and bacteremia, mainly in patients with serious underlying conditions or immunosuppression (2, 22, 25). Systemic C. fetus subsp. fetus infections often require prolonged parenteral therapy with one or two antibiotics, and the prognosis may be poor for the compromised host (2). There is no standardized susceptibility testing method for this organism (19).

The objectives of this study were to determine β-lactamase production of 59 strains of C. fetus subsp. fetus isolated in Québec, Canada, and the antimicrobial susceptibility patterns of those strains against eight antibiotics. Three methods of susceptibility testing were compared: agar dilution, disk diffusion, and the E test (21).

Fifty-nine strains were tested by the agar dilution and the disk diffusion tests with eight antibiotics, 17 of which strains were isolated in our institution and 42 of which were provided by the Laboratoire de Santé Publique du Québec. Of the 59 strains, 30 were also tested by the E test for four of the antibiotics. All strains were previously identified as C. fetus subsp. fetus based on standard criteria and methodology (19).

The antibiotics used were ampicillin, gentamicin, erythromycin, tetracycline, ceftoxime, ciprofloxacin (all from Sigma Chemical Co., St. Louis, Mo.), imipenem (Merck Sharp & Dohme, West Point, Pa.), and meropenem (Zeneca Pharma Inc., Mississauga, Ontario, Canada).

Suspensions of 24- to 48-h blood agar cultures were adjusted to a 0.5 McFarland turbidity standard in Mueller-Hinton broth (BBL Microbiology Systems) for the disk diffusion and E test methods and diluted 1:10 for agar dilution. A final inoculum of 10³ CFU was applied to unsupplemented Mueller-Hinton plates (BBL) for agar dilution with a Cathra 3-mm replicator. The antibiotic concentrations ranged from 0.06 to 128 μg/ml, and a control plate without antibiotic was inoculated at the beginning of the procedure. All plates were incubated for 48 h at 35°C under microaerophilic conditions (about 5% O2, 10% CO2, and 85% N2) with gas generator envelopes (Difco).

A standard method of inoculation was used for disk diffusion and the E test (21) with the same medium and incubation conditions as for agar dilution.

For agar dilution, the endpoint was taken as the concentration that resulted in complete inhibition of visible growth. For the E test, the MIC was read at the point of intersection between the inhibition ellipse and the E-test strip. For the disk diffusion test, the antibiotic concentrations were chosen according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (21). The zone diameters were measured with slipping calipers. NCCLS susceptibility criteria were used to interpret the results for aerobic organisms (20, 21), since no such criteria are available for C. fetus subsp. fetus.

The following quality control strains were used: Staphylococcus aureus ATCC 29213, S. aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853.

A nitrocefin solution method in microwells was used for β-lactamase detection, as previously described by Lachance et al. (13). S. aureus ATCC 29213 and S. aureus ATCC 25923 were used as positive and negative controls, respectively. Three Campylobacter jejuni strains in which β-lactamase had previously been isolated and characterized were also used as positive controls (14).

All strains were β-lactamase negative. The percentages of susceptible strains determined by the three methods are indicated in Table 1 with the corresponding disk diameters for the susceptible organisms. MIC ranges, the MICs at which 50% of the isolates are inhibited (MIC50s) and MIC90s determined by the agar dilution and E-test methods are also indicated. All isolates were susceptible to ampicillin, gentamicin, imipenem, and meropenem by the three methods. The lowest MICs were obtained with gentamicin, imipenem, and meropenem.

There was complete agreement between the agar dilution and disk diffusion test results for tetracycline: 27% of isolates were resistant and 73% were susceptible by both methods.

The percentages of intermediate MICs were 61% for erythromycin, 2% for ciprofloxacin, and 10% for ceftoxime by the agar dilution method. When agar dilution and disk diffusion were compared, minor error rates were 61% with erythromycin, 12% with ceftoxime, and 2% with ciprofloxacin. There was no major or very major error with any of the antibiotics.

Overall agreement of MICs ± 1 log2 dilution between the agar dilution and the E-test method (data not shown) was
TABLE 1. Susceptibilities of 59 strains of C. fetus subsp. fetus as determined by agar dilution, disk diffusion, or E test

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Method</th>
<th>Breakpoint for susceptible strains(^b)</th>
<th>% Susceptible</th>
<th>Disk diameter for susceptible organism (mm)</th>
<th>MIC ((\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Agar dilution</td>
<td>≤8</td>
<td>100</td>
<td>0.25–8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td>≤8</td>
<td>100</td>
<td>0.5–2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥17</td>
<td>100</td>
<td>18–60</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Agar dilution</td>
<td>≤4</td>
<td>100</td>
<td>0.12–1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td>≤4</td>
<td>100</td>
<td>0.5–2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥15</td>
<td>100</td>
<td>27–48</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>Agar dilution</td>
<td>≤4</td>
<td>100</td>
<td>≤0.06</td>
<td>≤0.06</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td>≤4</td>
<td>100</td>
<td>0.03–0.25</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥16</td>
<td>100</td>
<td>41–74</td>
<td></td>
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<tr>
<td>Meropenem</td>
<td>Agar dilution</td>
<td>≤4</td>
<td>100</td>
<td>≤0.06–0.25</td>
<td>≤0.06</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td>≤4</td>
<td>100</td>
<td>0.03–0.5</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥14</td>
<td>100</td>
<td>36–54</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>Agar dilution</td>
<td>≤8</td>
<td>90</td>
<td>4–16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>E test</td>
<td>≤8</td>
<td>0</td>
<td>16–64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥23</td>
<td>84</td>
<td>20–50</td>
<td></td>
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<tr>
<td>Erythromycin</td>
<td>Agar dilution</td>
<td>≤0.5</td>
<td>39</td>
<td>0.12–4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥23</td>
<td>100</td>
<td>32–54</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Agar dilution</td>
<td>≤4</td>
<td>73</td>
<td>≤0.06–128</td>
<td>≤0.06</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥19</td>
<td>73</td>
<td>36–71</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Agar dilution</td>
<td>≤1</td>
<td>98</td>
<td>≤0.06–2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Disk diffusion</td>
<td>≥21</td>
<td>100</td>
<td>23–46</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Breakpoints are those established by the NCCLS (20, 21) in micrograms per milliliter for agar dilution and the E test and in millimeters for disk diffusion for organisms that grow aerobically. The breakpoints of strains susceptible to meropenem are those established by Zeneca Pharma Inc.

\(^b\) Determined by agar dilution.

100% with gentamicin and imipenem, 98% with meropenem, and 90% with ampicillin. An important discrepancy was seen with cefotaxime, to which none of the 30 strains were susceptible by the E test, with 10 and 90% being intermediate and resistant, respectively (compared with 90% of strains being susceptible and 10% being intermediate by the agar dilution method). This was not observed with the control strains, for which MICs were within the expected limits under identical testing conditions. Also, such a difference was not observed between the agar dilution and the disk diffusion tests, whose results were more concordant (90% susceptible by agar dilution and 84% susceptible by disk diffusion).

Few studies of C. fetus subsp. fetus susceptibility have been published over the past 20 years, and in those few, a wide variety of inocula and media have been used (3–6, 9, 11, 12, 17, 26), mostly on a small number of strains (average, 13; range, 2 to 30). Interstudy comparison has therefore been difficult.

Fliegelman et al. (6) and Spelhaug et al. (26) evaluated β-lactamase production and found no positive strains (of a combined total of 34 for both studies), which is in agreement with our results. None of our isolates were resistant to ampicillin. The MIC range obtained was comparable to those in the literature describing tests in which a similar inoculum was used (6, 9, 17, 26). A small number of resistant strains were reported in studies (3–5) with inocula with McFarland standards ranging from 1 to 3, but none of these studies reported β-lactamase testing. Treatment failures have been reported in a recent review (16), but patients described in that work suffered from severe endovasculitis and associated conditions. Ampicillin has been considered a reasonable choice whenever susceptibility testing could be done (2, 7). In two studies (17, 26), its bactericidal activity was considered superior to those of expanded-spectrum cephalosporins, such as cefotaxime, but inferior to those of gentamicin and imipenem.

Similar to our findings, gentamicin (3–6, 9, 12, 17, 26), imipenem (17, 26), and meropenem (12) had the lowest MICs in previous studies, and no resistance has yet been reported. Gentamicin and imipenem are considered the preferred treatments for severe systemic infections (2, 9, 18), and imipenem was suggested as the first-choice agent for dealing with central nervous system infections (17).

Our results showed a high percentage of intermediate MICs of erythromycin. This was also previously observed (4–6, 9, 12) with C. fetus subsp. fetus. Several therapeutic failures or relapses were reported with that agent, sometimes with strains initially shown to be sensitive, and it is no longer a recommended treatment for systemic C. fetus subsp. fetus infections (2, 7, 16, 23).

Reported resistance to tetracycline has been low, occurring in one or two strains and in only one study (9), in contrast with our finding that 27% of our isolates were resistant to tetracycline. C. jejuni and Campylobacter coli tetracycline resistance mediated by a plasmid with demonstrated transmissibility from C. jejuni to C. fetus (29) has been described by Taylor et al. (27, 29).

Few of the reviewed studies reported ciprofloxacin resistance (9, 12, 24). C. fetus subsp. fetus quinolone resistance mediated by a mutation on the gyrase gene has been described.
Two cases of bacteremic infection with quinolone-resistant \textit{C. fetus} subsp. \textit{fetus} in immunosuppressed patients who had prior contact with the antibiotic have been reported (15).

While a majority of our isolates were sensitive to cefotaxime by the disk diffusion and the agar dilution methods, the MIC\textsubscript{50} and MIC\textsubscript{90} for the isolates were at the susceptibility limit of 8 \(\mu\)g/ml. Intermediate MIC\textsubscript{90} of cefotaxime were found in five studies (9, 11, 12, 17, 26), and its bactericidal activity was generally lower than those of ampicillin, gentamicin, and imipenem (17, 26). Although expanded-spectrum cephalosporins were mentioned as an alternative choice in treating \textit{C. fetus} subsp. \textit{fetus} systemic infections (2), we tend to believe that cefotaxime should be used with caution, keeping in mind that its activity could be only moderate. E-test susceptibility testing for that agent seems unreliable with \textit{C. fetus} subsp. \textit{fetus}, and we do not recommend its use until further study can be done.

Overall agreement among the three testing methods with the antimicrobial agents most often used clinically in treating \textit{C. fetus} subsp. \textit{fetus} infections (ampicillin, gentamicin, imipenem, and meropenem) was good. None of the MICs for tested isolates were intermediate or resistant, and had they been, it still cannot be concluded that results would have been comparable. However, the disk diffusion and E-test methods have been evaluated in comparison with agar dilution for other \textit{Campylobacter} species (1, 8, 10) of which resistant or intermediate strains were involved, and results showed a high percentage of agreement in each category for most of the antibiotics. The E-test and disk diffusion thus could be acceptable and convenient methods of susceptibility testing when agar dilution cannot be performed.

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REFERENCES