Antimicrobial Susceptibility of *Campylobacter fetus* subsp. *fetus* Isolated from Blood and Synovial Fluid

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*Campylobacter fetus* subsp. *fetus* is a rare human pathogen, but can cause serious extraintestinal infections. Effective antimicrobial agent is required for the therapy, but we have very limited knowledge on the susceptibility of the organism. In this study, the susceptibility of 25 isolates of the organism to 14 antimicrobial agents was tested by an agar dilution method. Antimicrobial agents with low MIC ranges, in µg/ml, were: meropenem ≤0.25, dirithromycin ≤0.5, gentamicin ≤1, amikacin, ofloxacin, tetracycline and erythromycin ≤2. The MIC range of cefepime was 0.5-8 µg/ml, but those of other β-lactams were relatively high. All of the isolates were interpreted to be susceptible to cefepime, meropenem, amikacin, gentamicin, ofloxacin, tetracycline and dirithromycin. A significant proportion of the isolates were either intermediate or resistant to ampicillin, cephalothin, cefotaxime, aztreonam, loracarbef and erythromycin. In conclusion, the organism remains susceptible to aminoglycosides and tetracycline. Greater in vitro activity of meropenem, ofloxacin and dirithromycin require clinical evaluation.

Key Words: *Campylobacter fetus* subsp. *fetus*, antimicrobial susceptibility, agar dilution test

*Campylobacter fetus* subsp. *fetus* causes extraintestinal infection, mostly in patients with underlying diseases such as liver cirrhosis, immunosuppression, neoplasm or damaged heart valve (Penner, 1988). But, the infection may also occur in pregnant women without any underlying disease (Hukumoto et al. 1988).

*C. fetus* subsp. *fetus* infection has wide clinical spectra which are mostly serious. Septicemia is the most common infection (Righter et al. 1983; Francioli et al. 1985), but the infection includes meningitis (Rao et al. 1987; Inoue et al. 1993), vascular infection (Chong et al. 1970; Carbone et al. 1985; Righter & Wood, 1985), spontaneous peritonitis (Targan et al. 1976), pyogenic arthritis (Lim et al. 1990; Yao et al. 1993) and abortion (Gribble et al. 1981; Simor et al. 1986).

Because of the frequent recurrence of the infection and relatively high mortality of the patients (Carbone et al. 1985), treatment of the patients with the most active antimicrobial agent is desirable. As the susceptibility of each isolate can not be determined easily due to the fastidious growth requirement of the organism, empirical selection of drugs are inevitable.

However, we have very limited knowledge on the susceptibility of the organism (Taylor et al. 1988; Morrison et al. 1990). Moreover, most of the literature are old and were based
Susceptibility of *C. fetus* subsp. *fetus*

on small number of isolates in other countries (Butzler et al. 1974; Chow et al. 1978; Karmali et al. 1980; Spelhag et al. 1981; Edmonds et al. 1985; Fliegelman et al. 1985; Morooka et al. 1989). Emergence of resistance to some antimicrobial agents such as erythromycin was reported (Chow et al. 1978), while we are not aware of the susceptibility of the organism to newer drugs.

The purpose of this study was to determine the in vitro susceptibility of relatively recent isolates of *C. fetus* subsp. *fetus* to various antimicrobial agents including the new cephalosporin, carbapenem, carbacephem, quinolones and macrolide.

**MATERIALS AND METHODS**

Strains of *C. fetus* subsp. *fetus* used in this study were isolated: 13 of them in 1980s and 12 in 1990s: 22 from patients at Severance Hospital and 3 at other hospitals; 23 from blood and one each from synovial fluid of hip joint and elbow joint.

The criteria used for species identification included gram-negative curved bacilli, positive tests for oxidase, catalase and nitrate reduction, negative tests for hydrogen sulfide production in triple sugar iron agar and hippurate hydrolysis, cephalothin susceptibility and nalidixic acid resistance by disk test, and no growth at 42°C (Penner, 1988). Until used for the test, the isolates were either kept frozen in 20% skim milk or at room temperature in semisolid medium tubes prepared by combining SIM (Difco) and Brewer thioglycollate medium (Difco).

Antimicrobial susceptibility was determined by an agar dilution method of the National Committee for Clinical Laboratory Standards (NCCLS, 1992) using Mueller-Hinton agar (Difco) supplemented with 5% defibrinated rabbit blood. Antimicrobial agents used were ampicillin, cephalothin, erythromycin and tetracycline (Sigma Chemical, St. Louis, Mo., USA), cefotaxime (Handok, Seoul), cefepime and aztreonam (Bristol-Myers Squibb, Princeton, N.J., USA), meropenem (Sumitomo Pharmaceutical, Tokyo, Japan), loracarbef and dirithromycin (Eli Lilly, Indianapolis, In., USA), amikacin (Dong-A, Seoul), gentamicin (Dongwha, Seoul), ciprofloxacin (Miles Pharmaceutical, West Haven, Conn., USA) and ofloxacin (Daiichi Pharmaceutical, Tokyo, Japan).

Inocula were prepared in saline by suspending the colonies developed on 48-hour incubated blood agar. The turbidity was adjusted to match that of a McFarland No. 0.5 tube. Antimicrobial agent-containing plates were inoculated with a Steers replicator (Craft Machine Inc., Chester, Pa., USA). The plates were incubated at 35°C for 48 hours in anaerobic jars filled with mixed gas of 90% nitrogen, 5% hydrogen and 5% carbon dioxide. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as control organisms.

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent which inhibited visible growth. NCCLS breakpoints (NCCLS, 1992) were applied to interpret the MICs. Cefepime MIC of ≤8 μg/ml, 16 μg/ml and ≥32 μg/ml were interpreted as susceptible, intermediate and resistant, respectively (Fung-Tomc et al. 1989) and erythromycin breakpoint was used for dirithromycin, for which the NCCLS breakpoints were not available.

To compare the results of the agar dilution and the disk diffusion methods, strains inhibited by ≥16 μg/ml of cephalothin were tested by the disk diffusion method using 30 μg cephalothin disk and blood agar. The same inocula and incubation condition were used for both tests as described above.

**RESULTS**

The MIC range of meropenem was the lowest, ≤0.008-0.25 μg/ml against the isolates of *C. fetus* subsp. *fetus* (Table 1). Other antimicrobial agents with relatively low MIC ranges were amikacin 0.5-2 μg/ml, gentamicin ≤0.06-1 μg/ml, ciprofloxacin 0.25-4 μg/ml, ofloxacin 0.12-2 μg/ml, tetracycline 0.5-2 μg/ml, erythromycin 1-2 μg/ml and dirithromycin 0.12-0.5 μg/ml. Among the β-lactam antibiotics, the MIC ranges of ampicillin and cefepime were relatively
Table 1. Activities of antimicrobial agents against *C. fetus* subsp. *fetus* 

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml)</th>
<th>% of isolates:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.5-16</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>8-128</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.5-8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>4-32</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>2-128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Loracarbef</td>
<td>0.5-32</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤0.008-0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0.5-2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤0.06-1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25-4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0.12-2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5-2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1-2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Diritromycin</td>
<td>0.12-0.5</td>
<td>0.25</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* a No. of isolates tested were 25.  
* b Based on NCCLS breakpoints (1992) with exception for cefepime and dirithromycin. MIC of cefepime were interpreted ≤8 μg/ml as susceptible and ≤32 μg/ml as resistant. For dirithromycin, erythromycin breakpoints were used.

low, 0.5-16 μg/ml and 0.5-8 μg/ml, respectively, while those of loracarbef and cefotaxime were relatively high, 0.5-32 μg/ml and 4-32 μg/ml, respectively. The MIC ranges of cephalothin and aztreonam were much higher, 8-128 μg/ml and 2-128 μg/ml, respectively.

When the MICs for 90% of the isolates (MIC90) were compared, meropenem was the lowest, 0.25 μg/ml and dirithromycin was 0.5 μg/ml. The MIC90 of cefepime, amikacin, gentamicin, ciprofloxacin, ofloxacin, tetracycline and erythromycin were ≤4 μg/ml, and the MIC90 of cephalothin and aztreonam were ≥128 μg/ml.

It was interpreted that all of the isolates were susceptible to cefepime, meropenem, amikacin, gentamicin, ofloxacin, tetracycline and dirithromycin. However, the following resistance rates were noted: 20% to loracarbef, 28% to cefotaxime, 88% to cephalothin and 96% to aztreonam. All of the isolates were intermediate to erythromycin and 12% to ampicillin.

Among the 23 isolates compared, six (26%) were resistant to cephalothin by both agar dilution and disk diffusion methods (Fig. 1).

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**Fig. 1. Relation of minimal inhibitory concentration of cephalothin and inhibition zone diameter by 30 μg disk of cephalothin, and of the interpretations (Abbreviations: R, resistant; I, intermediate; S, susceptible).**

However, among the 16 isolates, resistant by the agar dilution method, eight (35%) each of them were intermediate and susceptible, respectively, by the disk diffusion method.
Susceptibility of *C. fetus* subsp. *fetus*

One isolate (4%), intermediate by the dilution method, was susceptible by the diffusion method and the inhibition zone diameter was very large, i.e., 32 mm.

**DISCUSSION**

Members of the genus *Campylobacter* are causative agents of both animal and human infections. Among the various species, *C. jejuni* and *C. coli* commonly cause enteritis in otherwise healthy persons (Penner, 1988). *C. fetus* subsp. *fetus* infection is less common. Since the first isolation of the organism in 1970 in Korea (Chong et al. 1970), we noted only 22 bacteriologically proven cases in the literature (Chong et al. 1979; Lee et al. 1981; Lee et al. 1983; Kim et al. 1986; Lee et al. 1987; Woo et al. 1987; Shin et al. 1988; Lim et al. 1990; Song et al. 1992). However, the infection should be more prevalent considering these facts: some people prefer raw calf liver dish which may contain the organism (Hukunoto et al. 1988), detection of the infection is not always possible because of the growth requirement of enriched media, a microaerophilic condition and a long incubation time (Penner, 1988), and unreported cases may not be small in number.

*C. fetus* subsp. *fetus* infection presents a variety of clinical manifestations, including severe ones. Recommended drugs were gentamicin, tetracycline and erythromycin for the bacteremia, and chloramphenicol for the meningitis (Morrison et al. 1990). Depending on infections, prolonged therapy was considered necessary (Richter et al. 1983; Francioli et al. 1985). Success of antimicrobial therapy depends on the susceptibility of the infecting organism. However, even if *C. fetus* subsp. *fetus* is isolated, accurate determination of the susceptibility is difficult because of the fastidious growth requirement. At present there is no guideline by the National Committee for Clinical Laboratory Standards of the U. S. on the susceptibility testing for this organism.

It was difficult to compare in vitro susceptibility of *C. fetus* subsp. *fetus* because various procedures were used depending on the investigators. Chow et al. (1978) used Mueller-Hinton agar with 5% defibrinated sheep blood, while Fliegelman et al. (1985) used Wilkins-Chalgren agar. Inocula varied from the turbidity of McFarland No. 3 tube to a 1:10 dilution of that of No. 0.5 tube. We used Mueller-Hinton agar with 5% defibrinated rabbit blood and the inoculum was adjusted to a McFarland No. 0.5 tube.

We were unable to find any previous report on the activity of meropenem against *C. fetus* subsp. *fetus*. In our result, the MIC range of meropenem was the lowest, ≤0.008-0.25 μg/ml, which was similar to that of imipenem (Spelhaug et al. 1981; Morooka et al. 1989). It was reported that imipenem was more active in vitro than ampicillin, and that the combination of imipenem and gentamicin would be more effective in the treatment of endocarditis, and imipenem alone for the treatment of meningitis due to the organism.

Chow et al. (1978) reported that the MIC range of erythromycin was 0.2-25 μg/ml. However, all of our isolates were inhibited by 2 μg/ml, which was similar to the result of others (Butzler et al. 1974; Edmonds et al. 1985; Fliegelman et al. 1985). Our result may indicate that erythromycin remains effective for most of the infections. Lower MIC range of dirithromycin than that of erythromycin, in our study, suggests that the former may be more effective. Garcia-Rodriguez et al. (1989) considered that newer macrolides, such as dirithromycin and roxithromycin, were more promising agent than erythromycin against *Helicobacter pylori* which is another curved organism.

The MIC ranges of amikacin, gentamicin, tetracycline, against our isolates were ≤2 μg/ml, which were similar to those by others, indicating that the organism remains susceptible to these drugs. The MIC range of ciprofloxacin was ≤4 μg/ml, which was slightly higher than the result of Fliegelman et al. (1985), i.e., ≤1 μg/ml. We consider that slightly elevated MIC of ciprofloxacin but slightly lower value of ofloxacin, ≤2 μg/ml, require further study.

The activity of ampicillin was quite variable depending on the investigators, indicating presence of resistant strains. In our result, the
MIC range of ampicillin was 0.5-16 μg/ml. When the NCCLS breakpoint was applied, 12% of the strains were interpreted as intermediate. This result suggests that when ampicillin is used without susceptibility testing, treatment failure may result.

Generally, cephalosporins are not very active against Campylobacter sp., although the susceptibility of C. fetus subsp. fetus to 30 μg cephalothin disk is one of the criteria used to differentiate the organism from C. jejuni and C. coli (Karmali et al. 1980). In this study, the MIC of cephalothin was quite variable depending on the strains, i.e., 8-128 μg/ml. The notion that C. fetus subsp. fetus is susceptible to cephalothin should not be extended to the selection of the drug for the treatment, as most of the strains are resistant to cephalothin. It was reported that although the MIC of cephalothin against C. fetus subsp. fetus was significantly lower than that against C. jejuni, the value was not very low (Butzler et al. 1974; Chow et al. 1978; Fliegelman et al. 1985). Woo et al. (1987) reported that 2 of 8 strains tested by disk diffusion method were resistant to cephalothin. By the disk diffusion test, 39% of our isolates were susceptible, but among the 23 isolates tested by both methods, 8 of 9 isolates susceptible by the disk method were resistant by the agar dilution test, indicating unreliability of the disk method for C. fetus subsp. fetus. Slower growth of the organism may be an important factor affecting the accuracy of the disk diffusion test with all antimicrobial agents, but it can be assumed that discrepant results may be observed more often with relatively less active drugs.

It was interesting that all of our isolates were susceptible to cefepime, while 28% were resistant to cefotaxime when usual susceptible breakpoint of 8 μg/ml was applied. Four percent of our isolates were susceptible to cefotaxime, while 48% were susceptible to cefepime when breakpoint of 2 μg/ml were applied, which may be appropriate for central nervous system infection (Canton, 1993). Loracarbef and aztreonam were not very active. Morooka et al. (1989) also reported high MIC value of aztreonam against this organism.

It is concluded from the in vitro study that for the treatment of C. fetus subsp. fetus bacteremia, amikacin, gentamicin, or tetracycline may be used without in vitro susceptibility testing, while selection of ampicillin or cefotaxime requires in vitro testing. Cefepime may be more effective than cefotaxime for the treatment of central nervous system infection. Greater in vitro activity of meropenem, dirithromycin and ofloxacin noted in this study require further clinical evaluation.

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Number 3

319