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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

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on small number of isolates in other countries (Butzler *et al.* 1974; Chow *et al.* 1978; Karmali *et al.* 1980; Spelhaug *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℃ (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℃ 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. Activitis of antimicrobial agents against *C. fetus* subsp. *fetus*[a]

Antimicrobial agent	MIC (μg/ml)			% of isolates:[b]		
	Range	50%	90%	Susceptible	Intermediate	Resistant
Ampicillin	0.5-16	8	16	88	12	0
Cephalothin	8-128	64	128	4	8	88
Cefepime	0.5-8	2	4	100	0	0
Cefotaxime	4-32	16	32	20	52	28
Aztreonam	2->128	>128	>128	4	0	96
Loracarbef	0.5-32	16	32	40	40	20
Meropenem	≤0.008-0.25	0.25	0.25	100	0	0
Amikacin	0.5-2	1	2	100	0	0
Gentamicin	≤0.06-1	0.5	1	100	0	0
Ciprofloxacin	0.25-4	1	1	92	4	4
Ofloxacin	0.12-2	0.5	1	100	0	0
Tetracycline	0.5-2	1	2	100	0	0
Erythromycin	1-2	2	2	0	100	0
Dirithromycin	0.12-0.5	0.25	0.5	100	0	0

[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 (MIC_{90}) were compared, meropenem was the lowest, 0.25 μg/ml and dirithromycin was 0.5 μg/ml. The MIC_{90}s of cefepime, amikacin, gentamicin, ciprofloxacin, ofloxacin, tetracycline and erythromycin were ≤4 μg/ml, and the MIC_{90}s 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).

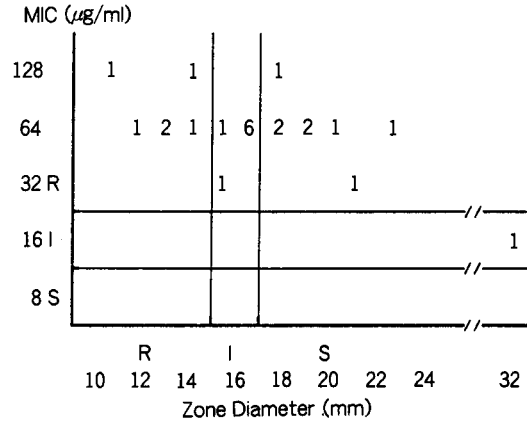

Fig. 1. *Relation of minimum inhibitory concentration of cephalothin and inhibition zone diameter by 30 μg disk of cephalothin, and of the interpretations (Abbreviations: R, resistant; I, intermediate; S, susceptible).*

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.

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 (Hukumoto *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 (Righter *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, $\leq$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 $\leq$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 $\leq$4 μg/ml, which was slightly higher than the result of Fliegelman *et al.* (1985), i.e., $\leq$1 μg/ml. We consider that slightly elevated MIC of ciprofloxacin but slightly lower value of ofloxacin, $\leq$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.

REFERENCES

Betzler JP, Dekeyser P, Lafontaine T: Susceptibility of related vibrios and *Vibrio fetus* to twelve antibiotics. *Antimicrob Agents Chemother 5: 86-89, 1974*

Canton E: Cefotaxime breakpoint for *Streptococcus pneumoniae. Antimicrob Agents Chemother 37: 616-617, 1993*

Carbone KM, Heinrich MC, Quinn TC: Thrombophlebitis and cellulitis due to *Campylobacter fetus* ssp. *fetus. Medicine 64: 244-250, 1985*

Chong Y, Kim YC, Lee SY, Moon YM: Two cases of *Campylobacter fetus* septicemia. *Yonsei Med J 20: 56-60, 1979*

Chong Y, Lee SY: *Vibrio fetus* human infection: Isolation from subacute bacterial endocarditis case. *Yonsei Med J 11: 126-130, 1970*

Chow AW, Patten V, Bednorz D: Susceptibility of *Campylobacter fetus* to twenty-two antimicrobial agents. *Antimicrob Agents Chemother 113: 416-418, 1978*

Edmonds P, Patton CM, Barrett TJ, Morris GK, Steigerwalt AG, Brenner DJ: Biochemical and genetic characteristics of atypical *Campylobacter fetus* subsp. *fetus* isolated from human in the United States. *J Clin Microbiol 21: 936-940, 1985*

Fliegelman RM, Petrak RM, Goodman LJ, Segreti J, Trenholme GM, Kaplan R: Comparative in vitro activities of twelve antimicrobial agents against *Campylobacter* species. *Antimicrob Agents Chemother 27: 429-430, 1985*

Francioli P, Herzstein J, Grob JP, Vallotton JJ, Mombelli G, Glauser MP: *Campylobacter fetus* subspecies *fetus* bacteremia. *Arch Intern Med 145: 289-292, 1985*

Fung-Tomc J, Dougherty TJ, DeOrio FJ, Smith-Jacobson V, Kessler RE: Activity of cefepime

against ceftazidime- and cefotaxime-resistant gram-negative bacteria and its relationship to β-lactamase levels. *Antimicrob Agents Chemother 33: 498-502, 1989*

Garcia-Rodriguez JA, Sanchez GJE, Garcia MIG, Sanchez EG, Bellido JLM: In vitro activities of new oral β-lactams and macrolides against *Campylobacter pylori. Antimicrob Agents Chemother 33: 1650-1651, 1989*

Gribble MJ, Salit IE, Isaac-Renton J, Chow AW: Campylobacter infection in pregnancy: A case report and literature review. *Am J Obstet Gynecol 140: 423-426, 1981*

Hukumoto A, Hori A, Matsubara I, Okamoto S, Nakajima S, Esaki T, Yabuuchi E: *Campylobacter fetus* subsp. *fetus* neonatal meningitis due to intrauterine infection. *Media Circle 33: 1-4, 1988*

Inoue Y, Ohtsubo T. Mori N, Ishino T, Takase T, Kaku M, Koga H, Kohno S, Hara K: A case of *Campylobacter fetus* subspecies *fetus* meningitis. *Kansenshogaku Zasshi 67: 66-70, 1993*

Karmali MA, DeGrandis S, Fleming PC: Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus* to eight cephalosporins with special reference to species differentiation. *Antimicrob Agents Chemother 18: 948-952, 1980*

Kim HJ, Yoon KJ, Chong Y, Lee SY, Han DS, Chun JY, Kim BS: *Campylobacter fetus* subsp. *fetus* septicemia: Report of four cases. *Kor J Clin Pathol 6: 57-62, 1986*

Lee KM, Koh YB, Chung IS: Endocarditis caused by *Campylobacter fetus* subsp. *intestinalis.* A case report. *Kor J Clin Pathol 3: 17-20, 1983*

Lee KW, Park AJ, Chong Y, Lee SY, Choi HJ: *Campylobacter fetus* subsp. *fetus* septicemia in a patient with liver cirrhosis and diabetes mellitus. *Kor J Pathol 15: 67-72, 1981*

Lee MH, Song KJ, Chong Y: *Campylobacter fetus* subspecies fetus septicemia in a patient with liver cirrhosis. *Kor J Clin Pathol 7: 87-90, 1987*

Lim HS, Chung HR, Chong Y, Lee SY, Han CD: Pyogenic hip joint due to *Campylobacter fetus* subsp. *fetus*: A case report. *Kor J Clin Pathol 10: 377-381, 1990*

Morooka T, Oda T, Shigeoka H: In vitro evaluation of antibiotics for treatment of meningitis caused by *Campylobacter fetus* subspecies *fetus. Ped Infect Dis J 8: 653-654, 1989*

Morrison VA, Lloyd BK, Chia JKS, Tuazon CU: Cardiovascular and bacteremic manifestations of *Campylobacter fetus* infection: Case report and review. *Rev Infect Dis 12: 387-392, 1990*

National Committee for Clinical Laboratory Standards: *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically,* 2nd ed. NCCLS, Villanova, Pa. *1992*

Penner JL: The genus *Campylobacter:* A decade of progress. *Clin Microbiol Rev 1: 157-172, 1988*

Righter J, Wells WA, Hart GD, McNeely DJ: Relapsing septicemia caused by *Campylobacter fetus* subsp. *fetus. Can Med Assoc J 128: 686-689, 1983*

Righter J, Wood JM: *Campylobacter* and endovascular lesion. *Can J Surg 28: 451-452, 1985*

Rao KV, Ralston RA: Meningitis due to *Campylobacter fetus intestinalis* in a kidney transplant recipient: A case report. *Am J Nephrol 7: 402-403, 1987*

Shin HJ, Jung HW, Kim EC, Chi JG: A case of *Campylobacter fetus* subdural empyema. *J Kor Neurosurg Soc 17: 807-813, 1988*

Simor AE, Karmali MA, Jadavji T, Roscoe M: Abortion and perinatal sepsis associated with *Campylobacter* infection. *Rev Infect Dis 8: 397-402, 1986*

Song WK, Park HS, Yoon KJ, Lee KW: A case of *Campylobacter fetus* subsp. *fetus* septicemia. *J Clin Pathol Quality Cintrol 14: 113-117, 1982*

Spelhaug DR, Gilchrist MJR, Washington JA: Bactericidal activity of antibiotics against *Campylobacter fetus* subspecies *intestinalis. J Infect Dis 143: 500, 1981*

Targan SR, Chow AW, Guze LB: Spontaneous peritonitis of cirrhosis due to *Campylobacter fetus. Gastroenterology 71: 311-313, 1976*

Taylor DE, Courvalin P: Mechanism of antibiotic resistance in *Campylobacter* species. *Antimicrob Agents Chemother 32: 1107-1112, 1988*

Woo JH, Choe KW, Kim EC: Clinical study on *Campylobacter fetus* sepsis. *Kor J Infect Dis 19: 251-259, 1987*

Yao JDC, Herman MC, Cambell I: Prosthetic hip joint infection due to *Campylobacter fetus. J Clin Microbiol 31: 3323-3324, 1993*