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Susceptibility Testing of *Campylobacter fetus* subsp. *jejuni*, Using Broth Microdilution Panels

GEORGE E. BUCK* AND MICHAEL T. KELLY

Microbiology Division, Clinical Laboratories, University of Texas Medical Branch, Galveston, Texas 77550

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Twenty-four isolates of *Campylobacter fetus* subsp. *jejuni* were tested by broth microdilution panels (Sensititre; GIBCO Diagnostics, Chagrin Falls, Ohio), and the minimal inhibitory concentrations (MICs) were compared with the corresponding MICs obtained by the standard agar dilution technique. Microdilution panels designed for testing gram-positive organisms were used so that erythromycin, the antibiotic of choice for this organism, could be included. The correlation with agar dilution was relatively poor when Mueller-Hinton broth was used; the MICs that were within one twofold dilution of the corresponding agar dilution MIC ranged from 15% with tetracycline to 75% with ampicillin. The overall agreement for all antibiotics tested was 48%. The correlation improved significantly, however, to an overall agreement of 87% when Wilkins-Chalgren broth was substituted in the broth microdilution procedure. Our results indicate that the broth microdilution test is an accurate method for testing this organism, provided that an appropriate medium is used.

Although enteritis caused by *Campylobacter fetus* subsp. *jejuni* is often a self-limited disease, many of the more serious cases require antibiotic treatment (4, 5). Studies have shown that this organism is generally susceptible to erythromycin, clindamycin, furazolidone, aminoglycosides, and tetracyclines (3, 10, 12), and at the present time erythromycin is considered the drug of choice (4, 5). However, up to 8% of isolates have been reported to be resistant to erythromycin (2, 12, 13), and tetracycline resistance has also been noted (1, 9, 11). Therefore, although susceptibility testing has generally not been advocated with this organism (4), there may be times when it would be worthwhile.

Currently, the only method for in vitro testing which has been used extensively is agar dilution, a method which is cumbersome for most laboratories to perform. Therefore, we investigated the possibility of using broth microdilution susceptibility panels for testing this organism. Our findings indicate that this method is quite satisfactory if an appropriate medium is used. The type of broth used appears to have a profound effect on the minimal inhibitory concentration (MIC) endpoint.

MATERIALS AND METHODS

Bacteria. Twenty-four isolates of *C. fetus* subsp. *jejuni* were used. They were isolated from stool specimens submitted to our laboratory, identified according to established criteria (8), and stored at −70°C in skim milk. For testing, the frozen stocks were cultured on fresh plates of 5% sheep blood agar at 37°C for 48 h in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. This was supplied by placing the plates in a Torbal anaerobic jar (Torsion Balance Co., Clifton, N.J.), evacuating the air as much as possible with the laboratory vacuum line, and then refilling with the gas mixture.

Agar dilution susceptibility test. The procedure used was a slight modification of standard agar dilution test (14). The organisms were grown on 5% sheep blood agar at 37°C for 48 h in a microaerophilic environment, and then colonies were removed from the plates and suspended in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) to the density of a McFarland no. 1 turbidity standard. In preliminary work, this produced approximately 10^8 colony-forming units per ml. The suspension was then diluted 1:10 in Trypticase soy broth, and a 0.001-ml calibrated loop was used to spot the suspension onto plates of Mueller-Hinton agar containing twofold concentrations of antibiotics. The concentrations selected for testing corresponded to the concentrations of the antibiotics used in the broth microdilution panels (see below). A control organism, either *Staphylcoccus aureus* (ATCC 25923) or *Escherichia coli* (ATCC 25922), was included on each plate. After inoculation, the plates were incubated at 37°C under microaerophilic conditions, generated as indicated above, and the results were read after 48 h. The endpoint used was complete inhibition of growth. The antibiotics used were as follows: ampicillin (Bristol Laboratories, Syracuse, N.Y.), cephalothin (Eli Lilly & Co., Indianapolis, Ind.), chloramphenicol (Parke, Davis & Co., Detroit, Mich.), clindamycin (The Upjohn Co., Kalamazoo, Mich.), erythromycin (Abbott Laboratories, North Chicago, Ill.), gentamicin (Schering Corp., Bloomfield, N.J.), kanamycin (Bristol Laboratories), penicillin (Eli Lilly & Co.), and tetracycline (Ames, Division of Miles Laboratories, Elkhart, Ind.).

TABLE 1. Results of agar dilution MIC testing

Antimicrobial agent	MIC range (μg/ml)	MIC_{50}[a] (μg/ml)	MIC_{90}[b] (μg/ml)
Ampicillin	2–>16	4	>16
Cephalothin	>64	>64	>64
Chloramphenicol	2–8	4	8
Clindamycin	0.25–2	0.5	2
Erythromycin	0.5–2	1	2
Gentamicin	≤0.12–1	0.5	0.5
Kanamycin	4–8	4	8
Penicillin	>8	>8	>8
Tetracycline	0.25–>16	0.5	>16

[a] MIC_{50}, Concentration required to inhibit 50% of test strain.

[b] MIC_{90}, Concentration required to inhibit 90% of test strain.

Broth dilution test. Lyophilized microdilution trays originally designed for use with gram-positive cocci (Sensititre no. AP01; GIBCO Diagnostics, Chagrin Falls, Ohio) were utilized. By using these panels, the important antibiotics for *C. fetus* subsp. *jejuni* could be tested, including erythromycin, which is not available on panels designed for gram-negative organisms. The bacteria were grown on 5% sheep blood agar, suspended in Trypticase soy broth to the density of a McFarland no. 1 turbidity standard and then diluted 1:100 in either Mueller-Hinton broth or Wilkins-Chalgren broth. The inoculum was then dispensed into the microdilution panels as indicated in the instructions supplied by the manufacturer. This produced a starting concentration of approximately 10^6 colony-forming units per ml. The trays were incubated at 37°C in a Torbal jar tipped on its side, with the microaerophilic atmosphere established as indicated above. Results were recorded after 48 h of incubation.

The composition of the Wilkins-Chalgren broth was as follows: tryptone, 10 g; peptone, 10 g; yeast extract, 5 g; dextrose, 1 g; sodium chloride, 5 g; arginine, 1 g; sodium pyruvate, 1 g; hemin, 5 mg; vitamin K1, 0.5 mg; water, 1 liter. The Mueller-Hinton broth used was the standard formula supplied with the Sensititre trays. It was not supplemented to obtain additional levels of Ca^{2+} and Mg^{2+}.

RESULTS

Our approach was to compare MICs obtained by the broth microdilution test to those obtained with agar dilution. The ranges of MICs and the concentrations of those drugs required to inhibit 50 and 90% of the 24 *Campylobacter* isolates by agar dilution are shown in Table 1. All of the isolates were susceptible to erythromycin, clindamycin, and gentamicin. They were moderately susceptible to chloramphenicol and kanamycin and highly resistant to penicillin and cephalothin. Although most isolates were quite susceptible to tetracycline, 3 of 24 were highly resistant (MICs > 16 μg/ml). The MICs for the control organisms (not shown) were within recommended limits (14), except for those of gentamicin, kanamycin, and erythromycin. With these three, the MICs for the control organisms were two dilutions higher than expected values, probably reflecting some degree of inactivation, which is known to occur with these antibiotics under increased CO_2 concentration (7).

The same organisms were then tested by broth microdilution with Mueller-Hinton broth. The correlation between MIC results obtained by the two methods is shown in Table 2. MICs that were "out of range" by either method, i.e., greater than the highest concentration tested or less than the lowest concentration tested, could not be compared directly and were excluded from the calculations. As a result, all of the MICs of penicillin and cephalothin were excluded, and the comparison is, therefore, based on the other antibiotics tested. The percent agreement between the two methods, i.e., the broth dilution MIC was within one dilution of the agar dilution MIC, ranged from 89% with gentamicin to 10% with clindamycin. The overall agreement was only 48%.

In search of an explanation for this poor correlation we noticed that many of the broth dilution MICs were much lower than the corre-

TABLE 2. Correlation of agar dilution with broth microdilution, using Mueller-Hinton broth

Antimicrobial agent	Broth MICs compared with agar MICs					
	No. comparable	Broth within one dilution of agar (no.)	Broth > one dilution higher (no.)	Broth > one dilution lower (no.)	% Agreement	No. not comparable[a]
Ampicillin	16	12	2	2	75	8
Chloramphenicol	19	12	0	7	63	5
Clindamycin	19	2	2	15	10	5
Erythromycin	16	6	1	9	38	8
Gentamicin	9	8	0	1	89	15
Kanamycin	19	11	0	8	58	5
Tetracycline	13	2	1	10	15	11

[a] MIC pairs in which either the broth or agar dilution MIC was beyond the highest concentration tested or less than the lowest concentration tested.

TABLE 3. Correlation of agar dilution with broth microdilution, using Wilkins-Chalgren broth

Antimicrobial agent	Broth MICs compared with agar MICs					
	No. comparable	Broth within one dilution of agar (no.)	Broth > one dilution higher (no.)	Broth > one dilution lower (no.)	% Agreement	No. not comparable[a]
Ampicillin	18	17	1	0	94	6
Chloramphenicol	24	20	0	4	83	0
Clindamycin	23	18	0	5	78	1
Erythromycin	23	20	0	3	87	1
Gentamicin	23	22	1	0	96	1
Kanamycin	24	22	0	2	92	0
Tetracycline	17	13	0	4	76	7

[a] MIC pairs in which either the broth or agar dilution MIC was beyond the highest concentration tested or less than the lowest concentration tested.

sponding agar dilution MICs. This, coupled with the fact that several of the strains did not grow at all in the broth dilution test, suggested that poor growth of the organism in Mueller-Hinton broth was responsible for the poor correlation. Therefore, the broth dilution tests were repeated with Wilkins-Chalgren broth, which has been suggested for susceptibility testing of *C. fetus* subsp. *jejuni* by other investigators (R. G. Streker and R. C. Tilton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C228, p. 312). When this was done, there were far fewer instances when the MICs could not be compared, and the correlation with agar dilution MICs was much better (Table 3). Gentamicin gave the best agreement (96%), and the poorest correlation occurred with tetracycline (76%). The overall agreement was 87%.

DISCUSSION

Four studies have been published concerning the antimicrobial susceptibility of *C. fetus* subsp. *jejuni* to a number of antibiotics (3, 10–12). In general, our results agreed with these previous findings. The organism was highly resistant to penicillin and cephalothin. Most isolates were moderately resistant to ampicillin, chloramphenicol, and kanamycin and susceptible to erythromycin, clindamycin, chloramphenicol, gentamicin, and tetracycline. Although erythromycin-resistant isolates have been reported in the literature (2, 10–13), none of our isolates was resistant to this antibiotic. However, 3 of the 24 isolates were resistant to tetracycline (MICs > 16 μg/ml).

Because the majority of isolates are susceptible to erythromycin, routine susceptibility testing has not been advocated for *C. fetus* subsp. *jejuni* (4, 5). Nevertheless, susceptibility testing may occasionally be necessary, such as in a suspected treatment failure, when treatment with an antibiotic other than erythromycin is being considered, or for an extraintestinal infection. This would be especially true in geographical areas where resistant isolates appear to be relatively common. Unfortunately, the only means of susceptibility testing used extensively so far is agar dilution, and this is difficult for many laboratories to use.

The results presented here show that the broth microdilution technique is acceptable if an appropriate medium is used. The accuracy of the test was only 48% when Mueller-Hinton broth was used, but it increased to 87% when Wilkins-Chalgren broth was substituted. This figure, although not especially high, may represent the maximum correlation possible between Sensititre plates and agar dilution, since other investigators obtained a similar figure with other organisms (6).

The poor correlation obtained with Mueller-Hinton broth appeared to be due to poor growth of the bacteria in this medium, although the reason for this is not entirely clear. It is also possible that other media, besides Wilkins-Chalgren broth, could be used. Additional work may help answer these questions. One advantage of using susceptibility panels which contain lyophilized antimicrobial agents is that other media can be substituted, whereas this cannot be done with frozen trays.

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