Comparison of Broth Microdilution, E Test, and Agar Dilution Methods for Antibiotic Susceptibility Testing of *Campylobacter jejuni* and *Campylobacter coli*

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A standardized broth microdilution method was compared to the E test and an agar dilution method for the antimicrobial susceptibility testing of *Campylobacter jejuni* and *C. coli* isolates. A group of 47 human clinical isolates, 37 isolates from retail poultry, and 29 isolates from living turkeys (total, 113 isolates) was included in the study. These encompassed 92 *C. jejuni* and 21 *C. coli* strains. The MICs of six antimicrobial agents were determined by the broth microdilution and E test methods, and the strains of human origin were additionally tested by the agar dilution method. In general, broth microdilution MICs agreed within 1 log2 MIC increment with 90.0% of E test results and 78.7% of agar dilution test results. The agar dilution method gave much lower gentamicin MICs than the broth microdilution method, but the data were significantly (*P < 0.01*) correlated and there was 100% agreement in the sensitivities and specificities in the comparison of the tests. The broth microdilution method had the highest sensitivity for analysis of the susceptibilities of *Campylobacter* to nalidixic acid and trimethoprim-sulfamethoxazole. The MICs of ciprofloxacin and erythromycin complied numerically by all three methods. The classification of the results and the correlation of the data demonstrated a high degree of agreement. All methods were equally suitable for the testing of the sensitivity of *Campylobacter* to tetracycline. Thus, the broth microdilution method appears to be an easy and reliable method for determination of the MICs of antibiotics for *C. jejuni* and *C. coli*, and it may offer an interesting alternative to MIC determination by the agar dilution technique or the E test.

Infections with *Campylobacter* species are one of the most common causes of bacterial diarrhea in humans worldwide, and the two species *Campylobacter jejuni* and *C. coli* are associated with the majority of infections in humans (20, 29). Most cases of *Campylobacter* enteritis do not require antimicrobial treatment, as they are of short duration, clinically mild, and self-limiting. However, antimicrobial treatment is necessary for patients with systemic *Campylobacter* infections, for immunosuppressed patients, and for patients with severe or long-lasting cases of *Campylobacter* enteritis.

Campylobacteriosis is a zoonotic disease; and domestic animals such as poultry, pigs, and cattle may act as reservoirs for *Campylobacter* isolates. As these isolates may be transferred from animals to humans via food, the emergence of antimicrobial resistance in enteric *Campylobacter* spp. due to the use of antimicrobial agents in husbandry is a matter of concern (23, 30). In order to monitor the prevalence of antimicrobial resistance of *Campylobacter* spp. isolated from food animals, food of animal origin, and humans in different laboratories, there is a need for standardized or intercalibrated methods (1, 9, 19).

*Campylobacter* spp. are fastidious bacteria requiring microaerophilic conditions for growth. Standardized procedures for susceptibility testing are available for a wide range of organisms, and in general, the guidelines provided by NCCLS (22) are the most widely used; however, no internationally accepted criteria for susceptibility testing of *Campylobacter* spp. are available and breakpoints do not exist. Consequently, a number of different diffusion and dilution methods have been used in clinical, veterinary, and food microbiology laboratories.

At present, susceptibility testing by the agar dilution method on plates supplemented with 5% blood and cultivated under microaerophilic conditions is most often recommended [1, 19; P. F. McDermott, S. M. Bodeis, and R. D. Walker, Abstr. 11th Int. Workshop *Campylobacter, Helicobacter* and Related Organisms, Int. J. Med. Microbiol. 291(Suppl. 31):109-110, 2001]. As the agar dilution method is very time-consuming, it is rarely performed in routine laboratories. The E test, a diffusion method with the ability to produce an MIC result (4), produces results more quickly, but it is saddled with problems with MIC testing under microaerophilic conditions for at least some substances (3, 9, 10, 13). Often, economic reasons limit the use of the E test for susceptibility testing in routine laboratories. The broth microdilution method, in comparison, is a fast and low-cost method for MIC determination. We recently standardized a broth microdilution method for susceptibility testing of *C. jejuni* and *C. coli* which is easy to perform, reasonable, and suitable for routine use [P. Luber, E. Bartelt, G. Klein, J. Wagner, and H. Hahn, Abstr. 11th Int. Workshop *Campylobacter, Helicobacter* and Related Organisms, Int. J. Med. Microbiol. 291(Suppl. 31):105, 2001].

The study reported herein was undertaken to confirm the usefulness of the standardized broth microdilution method for determination of the antibiotic susceptibility patterns of
examined for typical Campylobacter hydrolysis (24), hippurate hydrolysis, and susceptibility to nalidixic acid and indoxyl acetate. Each isolate from each sample was identified by the agar dilution method. Campylobacter colonies. One presumptive Campylobacter isolate from each sample was identified to the species level on the basis of phase-contrast microscopy (characteristic morphology and motility), Gram staining, catalase and oxidase production, growth at 25 and 43°C, indoxyl acetate hydrolysis (24), hippurate hydrolysis, and susceptibility to nalidixic acid and cephalothin. This procedure is in general accordance with guideline ISO 10272 of the International Organization for Standardization (14).

The 29 isolates from living turkeys were isolated from fecal samples taken from healthy animals in different German flocks (25) and submitted to BgVV for antimicrobial resistance testing in 2000. All isolates were stored at –80°C in a freezer by using the Microbank system (PRO-LAB Diagnostics, Cheshire, United Kingdom). C. jejuni ATCC 33560 and C. coli ATCC 33559 were used as control strains.

**Antimicrobial agents.** The broth microdilution test was performed with Sensititre susceptibility plates (MCS Diagnostics BV, Swalmen, The Netherlands). The E test strips were purchased from AB Biodisk (VIVA Diagnostica, Huerth, Germany). The antimicrobial powders for the agar dilution method, erythromycin and gentamicin, were purchased from Sigma (Deisenhofen, Germany), whereas ciprofloxacin was produced by Gruenenthal (Aachen, Germany), and trimethoprim-sulfamethoxazole was produced by Hoffmann-La Roche (Grenzach-Wyhlen, Germany).

An overview of the antimicrobial concentration ranges used in the tests is given in Table 1. The following NCCLS MIC interpretive standards for members of the family Enterobacteriaceae (for erythromycin, the MIC interpretive standard for Staphylococcus spp. was used) (21) were used as tentative breakpoints for the resistance of Campylobacter: for gentamicin and tetracycline, ≥16 μg/ml; for erythromycin, ≥8 μg/ml; for nalidixic acid, ≥32 μg/ml; for ciprofloxacin, ≥4 μg/ml; and for trimethoprim-sulfamethoxazole, ≥80 μg/ml.

**Antimicrobial susceptibility testing.** The broth microdilution test and the E test were performed in the BgVV laboratories for all isolates, whereas the agar dilution method was performed at the Benjamin Franklin Medical Center.

**Materials and methods.** Bacterial isolates. Forty-seven human clinical isolates, 30 isolates from retail chickens, 7 isolates from retail turkeys, and 29 isolates from living turkeys (total, 113 isolates) were included in the study. These encompassed 92 C. jejuni strains and 21 C. coli strains. Human isolates originated from patients with diarrhea; stool specimens from these patients had been submitted to the Benjamin Franklin Medical Center for clinical examination in 1998 and 1999, and the organisms were isolated there by standard laboratory methods (16). The isolates of poultry origin were isolated in previous studies and were randomly collected from the Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV) stock culture collection. The poultry meat samples were collected from retail stores in the Berlin region during the years 1991, 1998, and 2000. Thermophile Campylobacter spp. have been isolated from poultry meat samples since 1991 by selective enrichment in Preston broth (5) for 18 to 24 h at 42°C in a microaerophilic atmosphere (approximately 5% O2, 10% CO2, and 85% N2) that was created by a gas evacuation procedure (reducing the atmospheric pressure and regassing with a mixture of 80% nitrogen and 20% carbon dioxide). One loopful (10 μl) of the broth was transferred to Preston agar (CM 67 plus selective supplements SR 117 and SR 48; Oxoid GmbH, Wesel, Germany). The agar plates were incubated at 42°C for 2 days in a microaerophilic atmosphere and examined for typical Campylobacter colonies. One presumptive Campylobacter isolate from each sample was identified to the species level on the basis of phase-contrast microscopy (characteristic morphology and motility), Gram staining, catalase and oxidase production, growth at 25 and 43°C, indoxyl acetate hydrolysis (24), hippurate hydrolysis, and susceptibility to nalidixic acid and ciprofloxacin.

Ciprofloxacin ≥4 Broth microdilution (113) 0.008–16 0.06–16 16.0 0.25
E test (112) 0.002–32 0.03–32 16.0 0.12
Agar dilution (47) 0.007–8 0.03–8 16.0 0.12
Erythromycin ≥8 Broth microdilution (113) 0.008–16 0.25–16 32.0 1.0
E test (113) 0.016–256 0.12–256 32.0 1.0
Agar dilution (47) 0.015–16 0.5–16 32.0 1.0
Gentamicin ≥16 Broth microdilution (113) 0.015–32 0.12–2.0 64.0 1.0
E test (113) 0.016–256 0.25–2.0 64.0 1.0
Agar dilution (47) 0.015–16 0.06–0.5 64.0 0.25
Tetracycline ≥16 Broth microdilution (113) 0.008–16 0.06–16 32.0 32.0
E test (113) 0.016–256 0.03–256 32.0 32.0
Agar dilution (47) 0.007–8 0.06–8 16.0 16.0
Trimethoprim-sulfamethoxazole ≥80 Broth microdilution (113) 0.3–320 10.0–320 320 80.0
Agar dilution (46) 2.5–40 5.0–40.0 80.0 40.0
Nalidixic acid ≥32 Broth microdilution (113) 0.015–32 2.0–32.0 64.0 4.0
E test (113) 0.016–256 1.0–256 64.0 2.0

**TABLE 1. Overview of the antimicrobial agents, tentative breakpoints, methods, and antimicrobial concentration ranges included in the study plus the range and mode of MICs for 113 and 47 Campylobacter isolates, respectively, obtained by three different methods**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Tentative breakpoint (µg/ml) for resistance of Campylobacter spp.</th>
<th>Method* (no. of isolates tested)</th>
<th>Antimicrobial concn range (µg/ml)</th>
<th>MIC range (µg/ml)</th>
<th>Upper MIC limit for test comparison (µg/ml)*</th>
<th>Mode MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≥4</td>
<td>Broth microdilution (113)</td>
<td>0.008–16</td>
<td>0.06–16</td>
<td>16.0</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E test (112)</td>
<td>0.002–32</td>
<td>0.03–32</td>
<td>16.0</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar dilution (47)</td>
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<td>0.03–8</td>
<td>16.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≥8</td>
<td>Broth microdilution (113)</td>
<td>0.008–16</td>
<td>0.25–16</td>
<td>32.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E test (113)</td>
<td>0.016–256</td>
<td>0.12–256</td>
<td>32.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar dilution (47)</td>
<td>0.015–16</td>
<td>0.5–16</td>
<td>32.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥16</td>
<td>Broth microdilution (113)</td>
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<td>0.12–2.0</td>
<td>64.0</td>
<td>1.0</td>
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<td>E test (113)</td>
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<td>0.25–2.0</td>
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<td>Agar dilution (47)</td>
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<td>Broth microdilution (113)</td>
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<td>0.06–16</td>
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<td></td>
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<td>E test (113)</td>
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<td>0.03–256</td>
<td>32.0</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar dilution (47)</td>
<td>0.007–8</td>
<td>0.06–8</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≥80</td>
<td>Broth microdilution (113)</td>
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<td>10.0–320</td>
<td>320</td>
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<tr>
<td></td>
<td></td>
<td>Agar dilution (46)</td>
<td>2.5–40</td>
<td>5.0–40.0</td>
<td>80.0</td>
<td>40.0</td>
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<tr>
<td>Nalidixic acid</td>
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<td>2.0–32.0</td>
<td>64.0</td>
<td>4.0</td>
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<tr>
<td></td>
<td></td>
<td>E test (113)</td>
<td>0.016–256</td>
<td>1.0–256</td>
<td>64.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* The broth microdilution method and the E test were performed in the BgVV laboratories, whereas the agar dilution method was performed at the Benjamin Franklin Medical Center.

**To make the results from tests with different MIC scales comparable, an upper MIC limit was set, and data exceeding or remaining under this limit were transformed (lowered or enhanced).**
sphere consisting of 5% O2, 10% CO2, and 85% N2. For preparation of the test inoculum, 0.15 ml of this preculture was transferred to 10 ml of Mueller-Hinton broth, resulting in a suspension of 6 to 7 log CFU/ml (the number of CFU per milliliter for each inoculum was confirmed by culture). Further adjustment, e.g., to a turbidity standard of 1.0 McFarland, is not feasible, as described previously (Luber et al. Abstr. 11th Int. Workshop Campylobacter, Helicobacter and Related Organisms). Each well of the Sensititre microtiter plates for susceptibility testing was filled with 100 µl of suspension, and the plates were sealed with an anaerobic and microaerophilic film (Sensititre JPD MOS660) and incubated in the MACS VA500 incubator at 37°C under microaerophilic conditions. The test results were evaluated 24 h later. A positive control was included in each batch of broth microdilution tests. The MIC quality control limits for C. jejuni ATCC 33560 are as follows: for erythromycin, 1.0 to 4.0 µg/ml; for gentamicin, 1.0 to 4.0 µg/ml; for nalidixic acid, 4.0 to 16.0 µg/ml; for ciprofloxacin, 0.125 to 0.5 µg/ml; for tetracycline, 0.5 to 2.0 µg/ml; and for trimethoprim-sulfamethoxazole, 4.0 to 16.0 µg/ml. In the case of C. coli ATCC 35559, the MICs must be in the following ranges: for erythromycin, 2.0 to 8.0 µg/ml; for gentamicin, 0.25 to 1.0 µg/ml; for nalidixic acid, 4.0 to 16.0 µg/ml; for ciprofloxacin, 0.5 to 2.0 µg/ml; for tetracycline, 0.5 to 2.0 µg/ml; and for trimethoprim-sulfamethoxazole, 20.0 to 80.0 µg/ml. Each lot of Mueller-Hinton broth was controlled according to the NCCLS ATCC 29212, test values are expressed on a continuous scale, they were recoded in a log2 base elliptical zone of inhibition intersected with the MIC scale on the strip. Since E from the test strip according to the instructions of the manufacturer, where the suspension was plated on a Mueller-Hinton agar plate with 5% sheep blood.

in an ampoule of liquid medium. A total of 0.1 ml of the 1.0 McFarland suspension was plated on a Mueller-Hinton agar plate with 5% sheep blood. When the surface of each plate had dried, one E test strip was put on each plate. The plates were incubated with the lid side up at 37°C for 24 h in a MACS VA500 incubator, which created a microaerophilic atmosphere. MICs were read directly from the test strip according to the instructions of the manufacturer, where the elliptical zone of inhibition intersected with the MIC scale on the strip. Since E test values are expressed on a continuous scale, they were recoded in a log2 base scale for the comparison of the methods. E test MICs falling in the intermediate range of the log2 scale were rounded up to the higher value.

**Agar dilution test.** The agar dilution test was performed with Schaedler agar (CM 437; Oxoid) supplemented with 5% horse blood, and MIC testing was performed as described previously (2). The inoculum was applied with a Steers multipoint inoculator and was approximately 4 to 5 log CFU per spot. The agar plates were incubated in an anaerobic jar for 36 h at 37°C in a microaerophilic atmosphere. The MICs were calculated for each chemotherapeutic agent and method. In addition, Table 1 gives the log2 MICs for the 113 or 47 Campylobacter isolates tested, which resulted in a mode MIC 1 log2 step higher (Table 1). As shown by the classification results presented in Table 2, the broth microdilution method identified the same number of resistant isolates as the agar dilution method, and for the larger subset of strains tested by the broth microdilution method and the E test (n = 112), the broth microdilution method identified one more resistant strain than the E test. The agreement between the broth microdilution method and the E test was still 99%, and the kappa index indicated nearly complete concordance of the results of the two tests. The Kruskal-Wallis test showed no significant differences between the three methods (n = 47; P > 0.05), and the correlation of the results of the broth microdilution test with those of the E test and the agar dilution method was highly significant (P < 0.01).

**RESULTS**

**Ciprofloxacin.** The broth microdilution method tended to give slightly higher MICs for the *Campylobacter* isolates tested, which resulted in a mode MIC 1 log2 step higher (Table 1). As shown by the classification results presented in Table 2, the broth microdilution method identified the same number of resistant isolates as the agar dilution method, and for the larger subset of strains tested by the broth microdilution method and the E test (n = 112), the broth microdilution method identified one more resistant strain than the E test. The agreement between the broth microdilution method and the E test was still 99%, and the kappa index indicated nearly complete concordance of the results of the two tests. The Kruskal-Wallis test showed no significant differences between the three methods (n = 47; P > 0.05), and the correlation of the results of the broth microdilution test with those of the E test and the agar dilution method was highly significant (P < 0.01).

**Erythromycin.** The central tendency and the dispersion of the MICs for the 113 or 47 *Campylobacter* strains evaluated by the three methods were comparable. All tests identified the same *Campylobacter* isolates as resistant or sensitive (Table 2). Analysis of the degree of agreement between the three methods showed significant differences (n = 47; P < 0.01). However, the difference was less significant (n = 113; P < 0.05) between the broth microdilution method and the E test. The broth microdilution method provided lower log2 MICs than the other two methods for erythromycin. Nevertheless, the correlation of the MIC results obtained by the broth microdilution method, the E test, and the agar dilution method was highly significant (P < 0.01).

**Gentamicin.** All isolates tested were sensitive to gentamicin in vitro, which resulted in 100% agreement of the sensitivities and specificities in the comparison of the tests. The MICs of aminoglycosides are known to be influenced by the concentrations of cations in the medium (22). Presumably because of a different medium composition, the agar dilution method always produced lower MICs for the strains tested, which is displayed as mode concentrations 2 log2 steps lower (Table 1). Therefore, the Kruskal-Wallis test gave significant differences (n = 47; P < 0.01) for the degree of agreement between the three methods, but analysis of the relation between the results of the broth microdilution method and those of the E test only (by the Mann-Whitney test) displayed no differences in the results (n = 113; P > 0.05). The correlation of the MIC results by the broth microdilution method with those of the E test and
the agar dilution method was still highly significant ($P < 0.01$), as indicated by the Spearman correlation coefficients presented in Table 2, but there was no significant correlation at all between the results of the E test and those of the agar dilution method.

Tetracycline. The highest tetracycline concentration available for sensitivity testing by the agar dilution method was only 8 $\mu$g/ml, whereas the tentative breakpoint for *Campylobacter* resistance to tetracycline is $\geq 16$ $\mu$g/ml. The sensitivities of the broth microdilution method and the agar dilution method were 30% each (Table 2), but there was only 91% agreement in the identification of resistant isolates. The concordance was strong, as indicated by a kappa value of 0.797. Comparison of the results of the broth microdilution method and those of the E test displayed 98% agreement in the identification of resistant isolates and nearly complete concordance. Analysis of the number of times that each log$_2$ MIC of tetracycline occurred provided no significant differences between the three methods ($n = 47$; $P > 0.05$). Furthermore, no significant difference between the broth microdilution method and the E test ($n = 113$; $P > 0.05$) was obtained. The correlation of the MIC results by the broth microdilution method with those of the other two methods was highly significant ($P < 0.01$).

Trimethoprim-sulfamethoxazole. The sensitivity of *Campylobacter* to trimethoprim-sulfamethoxazole was tested by all three methods, but the E test offered test strips with an upper-limit concentration of only 32 $\mu$g/ml and trimethoprim-sulfamethoxazole MICs were $>32$ $\mu$g/ml for all 113 isolates tested by this method; therefore, only the broth microdilution method and the agar dilution methods were compared by use of trimethoprim-sulfamethoxazole. The highest concentration of trimethoprim-sulfamethoxazole in the agar dilution method was 40 $\mu$g/ml, whereas the tentative breakpoint for *Campylobacter* resistance to this agent is 80 $\mu$g/ml. Thus, isolates producing visible growth at a trimethoprim-sulfamethoxazole concentration of 40 $\mu$g/ml (>40 $\mu$g/ml) were classified as resistant (MIC = 80 $\mu$g/ml). The classification results for the broth microdilution method and the agar dilution method demonstrated different results for 19 of 46 isolates tested (Table 2). The agreement between the two methods was only 59%, and the kappa index indicated a weak concordance. The broth microdilution method identified 59% of the isolates as resistant, while the agar dilution test had a lower sensitivity and identified 39% of the isolates as resistant. The Mann-Whitney test found significant ($n = 46$; $P < 0.01$) differences in agreement between the two methods, because the log$_2$ MICs of trimethoprim-sulfamethoxazole were lower by the agar dilution method than by the broth microdilution method but the test results were significantly ($P < 0.05$) correlated.

**Nalidixic acid.** All 113 *Campylobacter* isolates were tested for their sensitivities to nalidixic acid by the broth microdilution method and the E test. As already indicated through the mode MIC and as clearly shown in Fig. 1, the E test tended to produce lower MICs than the broth microdilution method.

### Table 2. Classification results, test agreement, and correlation of MICs for each pair of test methods

<table>
<thead>
<tr>
<th>Methods compared and antimicrobial agent</th>
<th>No. of isolates with the following classification result</th>
<th>% Identification of resistant isolates by microdilution or E test</th>
<th>% Identification of resistant isolates by E test or agar dilution</th>
<th>% Agreement in identification of resistant isolates</th>
<th>Kappa</th>
<th>Correlation of MIC results</th>
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<tbody>
<tr>
<td>Broth microdilution method and E test</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>CIP</td>
<td>79 1 0 32</td>
<td>29</td>
<td>29</td>
<td>99</td>
<td>0.978</td>
<td>0.936**</td>
</tr>
<tr>
<td>ERY</td>
<td>106 0 0 6</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>1</td>
<td>0.718**</td>
</tr>
<tr>
<td>GEN</td>
<td>112 0 0 0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1</td>
<td>0.402**</td>
</tr>
<tr>
<td>TET</td>
<td>72 1 1 38</td>
<td>35</td>
<td>35</td>
<td>98</td>
<td>0.961</td>
<td>0.951**</td>
</tr>
<tr>
<td>SXT</td>
<td>78 2 0 32</td>
<td>30</td>
<td>29</td>
<td>98</td>
<td>0.957</td>
<td>0.908**</td>
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<tr>
<td>Broth microdilution and agar dilution methods</td>
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<td>CIP</td>
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<td>100</td>
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<td>0.872**</td>
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<tr>
<td>ERY</td>
<td>46 0 0 1</td>
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<td>2</td>
<td>100</td>
<td>1</td>
<td>0.617**</td>
</tr>
<tr>
<td>GEN</td>
<td>47 0 0 0</td>
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<td>0</td>
<td>100</td>
<td>1</td>
<td>0.396**</td>
</tr>
<tr>
<td>TET</td>
<td>31 2 2 12</td>
<td>30</td>
<td>30</td>
<td>91</td>
<td>0.797</td>
<td>0.914**</td>
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<tr>
<td>SXT</td>
<td>14 14 5 13</td>
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<td>39</td>
<td>59</td>
<td>0.204</td>
<td>0.369*</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>CIP</td>
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<td>30</td>
<td>30</td>
<td>100</td>
<td>1</td>
<td>0.883**</td>
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<tr>
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<td>2</td>
<td>100</td>
<td>1</td>
<td>0.532**</td>
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<tr>
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<td>28</td>
<td>93</td>
<td>0.843</td>
<td>0.884*</td>
</tr>
</tbody>
</table>

*a* CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; NAL, naladixic acid.

*b* For comparison of microdilution and E test methods, classification results signify the following: a, sensitive by both tests; b, resistant by microdilution and sensitive by E test; c, resistant by E test and sensitive by microdilution; d, resistant by both tests. For comparison of broth microdilution and agar dilution, classification results signify the following: a, sensitive by both tests; b, resistant by microdilution and sensitive by agar dilution; c, resistant by agar dilution and sensitive by microdilution; d, resistant by both tests. For comparison of E test and agar dilution, classification results signify the following: a, sensitive by both tests; b, resistant by E test and sensitive by agar dilution; c, resistant by agar dilution and sensitive by E test; d, resistant by both tests.

Kappa index values indicate concordance classification as follows: <0.01, no correlation; 0.1 to 1.40, weak correlation; 0.41 to 0.60, clear correlation; 0.61 to 0.80, strong correlation; 0.81 to 1.00, nearly complete correlation.

Values are Spearman correlation coefficients. *, significant correlation ($P < 0.05$); **, highly significant correlation ($P < 0.01$).
The broth microdilution method identified 34 isolates as resistant (test sensitivity = 30%), whereas the E test identified only 32 isolates as resistant (test sensitivity = 29%). The results of both methods had nearly complete concordance. The Mann-Whitney test demonstrated that the MICs obtained by the two methods differed significantly ($P < 0.01$), but the Spearman correlation coefficient was as high as 0.908, indicating a highly significant correlation ($P < 0.01$) of the MIC data.

**Broth microdilution method versus E test.** Table 3 displays the agreement of the MICs of five antimicrobial substances for 112 *Campylobacter* isolates generated by the broth microdilution method and the E test. The overall proportion of strains with results within an accuracy limit of $\pm 1 \log_2$ dilution was 90%. The levels of agreement between the two methods were high for erythromycin (95.9%), tetracycline (95.9%), and gentamicin (94.6%). Slightly lower levels of agreement were shown for the quinolones ciprofloxacin (88.4%) and nalidixic acid (75.9%) due to the tendency of the E test to produce lower MICs for this class of agents.

**Broth microdilution method versus agar dilution method.**

The percentages of strains yielding identical results by the broth microdilution and agar dilution methods were calculated for 47 *Campylobacter* strains and five antimicrobial agents (Table 4). The overall agreement was only 78.7% due to a very low level of agreement of the results for gentamicin (31.9%). The levels of agreement of the MICs of tetracycline (95.8%), erythromycin (93.6%), and ciprofloxacin (91.5%) were high, whereas the level of agreement was a bit lower for trimethoprim-sulfamethoxazole (80.5%). As in the comparison with the E test, differences were caused by the tendency of the broth microdilution method to produce higher MICs.

**DISCUSSION**

The broth microdilution method, the E test, and the agar dilution method overall produced comparable results, as indicated by the Spearman correlation coefficients (Table 2) and the percentages of strains yielding identical results by the broth microdilution method and the E test (Table 3) and by the broth microdilution method and the agar dilution method (Table 4). Commercially prepared microtiter plates and automated systems for MIC determination can be used for the broth microdilution method. Therefore, it is highly suitable for routine laboratories, and for the first time, this enables us to monitor the patterns of resistance of *Campylobacter* spp. to a greater extent.

The agar dilution method is the first method to have been standardized (McDermott et al., Abstr. 11th Int. Workshop *Campylobacter*, Helicobacter and Related Organisms) for MIC testing of *Campylobacter* isolates, and today it is the method recommended most often for *Campylobacter* sensitivity testing (1, 19). However, due to the large amount of manual handling needed to run the agar dilution test, it will never be used routinely. The E test already shows a faster performance than the agar dilution method, but again, the broth microdilution

**FIG. 1.** MIC histograms for 113 *Campylobacter* isolates tested for sensitivities to nalidixic acid by the E test and the broth microdilution method.
method is easier to handle. In comparison to the E test, the broth microdilution method is as technically simple, can be set up more rapidly, and can be automated. Furthermore, in previous studies (3, 13) the E test has shown limitations with regard to the antimicrobial agents with which *C. jejuni* could be tested. Thus, the choice of agents and the MIC ranges are limited when the variety of antibiotics that have clinical, diagnostic, and epidemiological relevance for thermophilic *Campylobacter* spp. are considered.

The broth microdilution method has occasionally been used in previous studies for antibiotic susceptibility testing of *Campylobacter* (8, 17, 27), but there was a lack of standardization of the method and many different broths and test parameters were used. One of the major points in standardization and quality control in *Campylobacter* susceptibility testing is the use of *Campylobacter* strains to verify the accuracy, precision, and reproducibility of test performance (7) in a microaerophilic atmosphere. NCCLS control strains for sensitivity testing such as *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 are not obligately microaerophilic and have previously been shown to be influenced by microaerophilic test conditions through pH alterations (Luber et al., Abstr. 11th Int. Workshop Campylobacter, Helicobacter and Related Organisms).

MIC data for a clinical *Campylobacter* strain that can be used to validate the outcomes of in vitro tests are lacking. Since an adequate gold standard for *Campylobacter* antibiotic sensitivity testing is not available and the true status of the isolates (resistant or sensitive) is not known, the sensitivity and specificity of a new test can be assessed only by comparison of the performance of the new method with that of another method (11). For a test evaluating the antibiotic sensitivities of bacteria, it is most important to identify resistance to an antibiotic.

The broth microdilution method had the highest sensitivity in analyzing the susceptibilities of *Campylobacter* isolates to nalidixic acid and trimethoprim-sulfamethoxazole (Table 2). For both agents, the broth microdilution method tended to give higher MICs. Figure 1 demonstrates the distributions of the MICs of nalidixic acid determined by the E test and the broth microdilution method. There were no differences in the sensitivities of the two methods when they were used to examine susceptibilities to ciprofloxacin, erythromycin, gentamicin, and tetracycline; but, with the exception of erythromycin, the broth microdilution method always tended to give slightly higher MICs, indicating its potency for the identification of resistant isolates. The tendency of the E test to produce lower MICs for *Campylobacter* spp. than the other test methods has been described previously (9, 13) for several substances.

Ciprofloxacin and erythromycin are the most commonly used drugs for the treatment of *Campylobacter* infections. For both agents, the broth microdilution method gave MICs that complied numerically with those obtained by the other two methods. The classification of the results and the correlation of the data demonstrated a high degree of agreement.

Emerging resistance to tetracycline, an agent which is often used in veterinary medicine (6), is a matter of concern for *Campylobacter* spp. (12, 18). There were some minor differences in the classification of isolates by the three methods due to the presence of some less susceptible *Campylobacter* strains for which the tetracycline MICs were near the tentative breakpoint. Considering the generally acceptable test limits of ±1 log dilution for each MIC, these results are within the range of accuracies of the test methods. All methods are equally suitable for testing the tetracycline sensitivities of *Campylobacter* strains, as supported by the very high percent agreements in Tables 3 and 4.

It is astonishing that for all strains included in the study the trimethoprim-sulfamethoxazole MIC by the E test was >32 μg/ml, whereas both the broth microdilution and the agar dilution methods gave MICs clearly lower for some of the strains. For the microaerophilic organisms *Haemophilus influenzae* and *Streptococcus pneumoniae*, problems have also been noted with the E test when it is used with trimethoprim-sulfamethoxazole under microaerophilic conditions (15).

In general, the data obtained by the three methods are probably biased because the tests were performed in two different laboratories. Further presumptive sources for deviation are the different origins of the antimicrobial agents used in the three methods and a possible divergence in the formulations of the agents, which might cause different activities. Moreover, the different medium used for the agar dilution method presumably had an effect on the test outcomes. The deviations of the agar dilution test results for gentamicin and trimethoprim-sulfamethoxazole might be based on this, as the MICs of amikaglycosides and sulfonamides are known to be influenced by the cation contents of the media or the end products of the folic acid cycle (22).

We had only a few technical difficulties with the broth microdilution method during our study. We observed a few trailing endpoints only with trimethoprim-sulfamethoxazole, as have been reported by other investigators when this agent is
used in the E test (13, 15). The MIC limits and tolerability ranges of the six antimicrobial agents which we formulated for the susceptibility testing of C. jejuni ATCC 33560 correspond to the tentative quality control ranges determined by the NCCLS VAST group for this type strain (McDermott et al., Abstr. 11th Int. Workshop Campylobacter, Helicobacter and Related Organisms). The performance of the broth microdilution method was very stable.

In summary, the usefulness of the broth microdilution method for determination of the antibiotic susceptibility patterns of Campylobacter spp. has been confirmed. The test is fast, can be easily set up, and is reasonably priced; and standardization can be achieved by using C. jejuni and C. coli type strains as controls. The broth microdilution method offers the opportunity to monitor the prevalence of resistance to specific drugs in Campylobacter isolates originating from food animals, food samples, and human infections to a great extent and may therefore help provide answers to questions concerning the emergence of antimicrobial resistance and the epidemiology of this important pathogen.

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