In Vitro Susceptibilities of Aerotolerant Campylobacter Isolates to 22 Antimicrobial Agents

JULIA A. KIEHLBAUCH,†‡ CAROLYN N. BAKER,‡ AND I. KAYE WACHSMUTH†

Enteric Diseases Branch,† Division of Bacterial and Mycotic Diseases, and Antimicrobics Investigations Branch,‡ Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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We evaluated the in vitro activities of 22 antimicrobial agents against 78 human and animal isolates belonging to two aerotolerant Campylobacter species, C. cryaerophila and C. butzleri, using a broth microdilution technique. An additional 10 antimicrobial agents were included at concentrations found in selective Campylobacter media. Strains of C. cryaerophila belonged to two DNA hybridization groups: DNA hybridization group 1A, which includes the type strain of C. cryaerophila, and DNA hybridization group 1B. The aminoglycosides, fluoroquinolones, and one tetracycline (minocycline) demonstrated the most activity against all DNA hybridization groups (C. cryaerophila DNA groups 1A and 1B and C. butzleri). Most isolates were resistant to cephalosporin antibiotics, with the exception of cefotaxime, and were variably susceptible to trimethoprim-sulfamethoxazole. C. cryaerophila DNA hybridization group 1A isolates were generally susceptible to the tetracyclines, chloramphenicol, nalidixic acid, azithromycin, erythromycin, and roxithromycin and moderately susceptible to clindamycin, trimethoprim-sulfamethoxazole, ampicillin, and ampicillin-sulbactam. The MICs of tetracyclines were higher for C. butzleri and C. cryaerophila DNA hybridization group 1B isolates than for C. cryaerophila DNA hybridization group 1A isolates, but most strains were still susceptible to doxycycline and tetracycline; all isolates were susceptible to minocycline. C. butzleri and C. cryaerophila DNA hybridization group 1B isolates were generally resistant to the macrolide antibiotics (including erythromycin), chloramphenicol, clindamycin, nalidixic acid, ampicillin, and trimethoprim-sulfamethoxazole. Differences in antimicrobial susceptibility between aerotolerant Campylobacter species and more common Campylobacter species, e.g., C. jejuni, suggest that different treatment strategies may be necessary. Strains of all three DNA hybridization groups of aerotolerant Campylobacter isolates were susceptible to colistin, polymyxin B, and rifampin at concentrations commonly used in selective media. These results suggest that primary isolation methods for Campylobacter species may need to be modified to include aerotolerant Campylobacter strains.

Previous investigations in our laboratory using DNA hybridization revealed two aerotolerant Campylobacter species, C. butzleri (previously designated DNA hybridization group 2) and C. cryaerophila (previously designated DNA hybridization group 1) (24). DNA hybridization data further separated strains of C. cryaerophila into two groups: DNA hybridization group 1A (containing the type strain of C. cryaerophila) and DNA hybridization group 1B. Two strains of DNA hybridization group 1B phenotypically resembled the type strain of C. cryaerophila (24). All strains from the United States belonged to C. butzleri and C. cryaerophila DNA hybridization group 1B; strains were most frequently isolated from fecal specimens from human and nonhuman primates with diarrheal illness (24, 39). C. cryaerophila DNA hybridization group 1A strains have been isolated only from animals in Ireland (24), whereas C. cryaerophila DNA hybridization group 1B contains three human strains from the United States and five animal isolates from Ireland. More recently, strains of C. cryaerophila DNA hybridization group 1B have been isolated from aborted porcine and equine fetuses in the United States (41). Other investigators have proposed that C. cryaerophila be renamed Arcobacter cryaerophilus. Under this proposal, C. butzleri would become Arcobacter butzleri (52). This study was designed to provide information regarding the antimicrobial susceptibilities of these aerotolerant Campylobacter organisms. Ten antimicrobial agents typically found in selective media were also included in this study to determine selective media that might be appropriate for primary isolation of these organisms. The third goal of this study was to determine whether antimicrobial susceptibility patterns could be used to differentiate these DNA hybridization groups, particularly those associated with human illness (C. butzleri and C. cryaerophila DNA hybridization group 1B).

MATERIALS AND METHODS

Bacterial isolates. A total of 78 isolates, belonging to the three DNA hybridization groups of aerotolerant Campylobacter isolates were included in this study. These isolates and their sources have been described in detail elsewhere (24). These isolates included 64 strains of C. butzleri (DNA hybridization group 2) and 14 C. cryaerophila isolates (6 isolates belonging to DNA hybridization group 1A and 8 strains belonging to DNA hybridization group 1B). Each isolate was stored at −70°C in tryptic soy broth containing 20% glycerol. Isolates were removed from the freezer and subcultured onto heart infusion agar containing 5% rabbit blood (BBL Microbiology Systems, Cockeysville, Md.), and plates were incubated at 30°C in an atmosphere of 5% O2, 7.5% CO2, 7.5% H2, and 80% N2. Organisms were subcultured one additional time prior to antimicrobial susceptibility testing.

* Corresponding author.
† Present address: Department of Pathology, Medical College of Wisconsin, Milwaukee, WI 53226.
Antimicrobial susceptibility test. The antimicrobial agents used are listed in Table 1. The drugs were diluted, as previously described (32), in cation-supplemented Mueller-Hinton broth with 5% lysed horse blood (in contrast to cation-adjusted Mueller-Hinton broth [33]), and dispensed into U-bottom microdilution trays. Quality control for the completed trays was done at the time of preparation by using Escherichia coli ATCC 25922 and ATCC 35218, Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, and Pseudomonas aeruginosa ATCC 27853. Trays were stored at −70°C until needed. In addition, control organisms were tested under the same incubation conditions (both microaerobic atmosphere and length of incubation) as the test organisms; all results were within acceptable limits. The trays were removed from the freezer and thawed at room temperature for at least 1 h prior to inoculation.

Growth from one plate of heart infusion agar containing 5% rabbit blood was harvested into Mueller-Hinton broth and adjusted to the turbidity of a MacFarland standard no. 0.5 (50). Incubation was performed by using a Dynatech Disposable Inoculator (Dynatech Laboratories, Inc. Alexandria, Va.), which provided a final inoculum of approximately 5 × 10^8 CFU/ml. At least one control strain was included with each run. Trays were incubated at 35°C in anaerobic jars (BBL) containing the atmosphere described above. Additional trays were inoculated with C. cryaerophila (DNA hybridization groups 1A and 1B) organisms, which were incubated at 30°C. Growth was recorded at 20 h and again at 48 h for those strains that did not exhibit satisfactory growth at 20 h. Results were interpreted by using published breakpoints for susceptibility and resistance for rapidly growing aerobic organisms (32); ampicillin results were interpreted using criteria for members of the family Enterobacteriaceae, azithromycin and roxithromycin breakpoints were interpreted according to erythromycin breakpoints, and ofloxacin breakpoints were interpreted as previously described (33).

Susceptibility of C. butzleri strains was determined after 20 h of incubation at 35°C; however, it was necessary to incubate C. cryaerophila strains for 48 h at 35°C.

RESULTS

The results of MIC testing of three hybridization groups of aerotolerant Campylobacter isolates are expressed in Table 1 as range, mode, MIC₅₀ (MIC for 50% of strains), and MIC₉₀ (MIC₉₀₅ are not listed for C. cryaerophila DNA hybridization groups because of the small number of isolates tested). The MICs for strains D2883 and D2884 are listed separately, because they do not clearly belong to either hybridization group. In general, C. cryaerophila DNA hybridization group 1A isolates were more susceptible to the agents tested than were C. butzleri and C. cryaerophila DNA hybridization group 1B isolates.

All 78 isolates tested were susceptible to minocycline, the aminoglycosides (amikacin and gentamicin), and the quinolones (ciprofloxacin, enoxacin, norfloxacin, and ofloxacin). The cephalosporins were the least active against aerotolerant Campylobacter strains; most strains were resistant to cephalexin, cefuroxime, and cefoperazone. Cefotaxime was the most active cephalosporin tested; however, 30% of C. butzleri isolates were resistant regardless of the cephalosporin. Variable susceptibility was noted for the macrolide antibiotics; most C. butzleri isolates included in this study were resistant to erythromycin (52%), azithromycin (75%), roxithromycin (95%), clindamycin (98%), and chloramphenicol (81%), whereas most C. cryaerophila (DNA hybridization groups 1A and 1B) isolates were intermediate susceptible to these antimicrobial agents. Resistance to ampicillin and ampicillin-sulbactam was common in C. butzleri isolates (83 and 69%, respectively) but less common in C. cryaerophila DNA hybridization group 1A (17% resistance to ampicillin). Many isolates were also resistant to trimethoprim-sulfamethoxazole; 33% of C. cryaerophila DNA hybridization group 1B isolates and 80% of C. butzleri isolates were resistant to this combination. Some isolates of C. butzleri and C. cryaerophila DNA hybridization group 1B were intermediately susceptible to doxycycline and tetracycline. Three concentrations of metronidazole were tested against aerotolerant Campylobacter isolates; C. cryaerophila DNA hybridization group 1A isolates did not grow in 4 µg of metronidazole per ml; however, 55% of C. butzleri isolates and all C. cryaerophila DNA hybridization group 1B isolates (except D2883 and D2884) grew at this concentration. Most C. butzleri isolates were susceptible to 32 and 64 µg of metronidazole per ml, whereas C. cryaerophila DNA hybridization group 1B isolates were generally susceptible to metronidazole only at 64 µg/ml.

An additional 10 antimicrobial agents were included at concentrations found in commercially available selective media for primary isolation of Campylobacter organisms. The susceptibilities of aerotolerant Campylobacter organisms to these agents are shown in Table 2. Aerotolerant Campylobacter organisms were resistant to amphotericin B, bacitracin, cefazolin, cycloheximide, novobiocin, trimethoprim, or vancomycin at concentrations found in selective media. Organisms were generally susceptible to polymyxin B and rifampin at concentrations used in selective media, and C. cryaerophila and most C. butzleri isolates were also susceptible to colistin.

C. cryaerophila DNA hybridization groups 1A and 1B, in addition to selected strains of C. butzleri, were tested in parallel at both 30 and 35°C. MICs obtained following incubation at both temperatures were generally within one dilution of each other. Strains that did not grow well overnight were incubated for 48 h, along with representative strains that did grow well. For strains that grew well, results were comparable at 20 and 48 h. Three subsets of organisms were present within C. butzleri, strains isolated from animals (n = 15), strains isolated from children in Thailand with diarrhea (n = 15), and strains of human origin isolated in the United States (n = 34). Comparison of the range, mode, MIC₅₀, and MIC₉₀ failed to reveal significant differences among the three subsets of C. butzleri organisms; the value for each differed by no more than two dilutions among the three groups (data not shown).

DISCUSSION

The antimicrobial susceptibility patterns of aerotolerant Campylobacter organisms found in this study were quite different from that previously described for other Campylobacter species. Aerotolerant Campylobacter strains associated with human illness appeared resistant to antimicrobial agents typically used in treatment of diarrheal illness caused by other Campylobacter species, e.g., erythromycin, other macrolide antibiotics, tetracycline, and chloramphenicol. In addition, aerotolerant Campylobacter isolates were typically resistant to clindamycin, a finding which has been previously described only for C. coli (25, 55). Increased MICs of erythromycin have been described only for isolates of C. coli (3, 8, 11, 14, 48, 55), C. fetus subsp. fetus (14), C. cinaedi (15), and animal strains of C. hyointestinalis (17). Human
strains of *C. hyointestinalis* (10), *C. jejuni*, *C. fennelliae* (15), *C. upsaliensis* (37), *C. fetus* subsp. *fetus* (14, 19, 30), and *C. lari* (31, 43, 47) are generally susceptible to erythromycin. Although the microaerobic atmosphere used in this study may decrease the level of activity of erythromycin and the aminoglycosides (7, 12, 40), this does not appear to have affected results in this study. In a similar study using the same conditions and media, 49 of 50 *C. jejuni* isolates had MICs to erythromycin ranging from 0.25 to 2.0 μg/ml (mode = 0.5 μg/ml) (2). This range is in accord with results established by other investigators.

In this study, the most active classes of antimicrobial agents against aerotolerant *Campylobacter* organisms were the aminoglycosides and quinolones in addition to minocycline. Other *Campylobacter* species are also generally susceptible to aminoglycosides (11, 14, 16, 26, 29, 42, 53) and the 4-fluoroquinolones (14, 18, 20, 26, 54). In contrast to other *Campylobacter* species, which demonstrate cross-resistance between nalidixic acid and other quinolones (1, 49), most strains of *C. fetus* and *C. cryaerophilia* DNA hybridization group 1B were resistant to nalidixic acid but not to other quinolones.

In addition, variable susceptibility was noted for trimethoprim-sulfamethoxazole, ampicillin, and ampicillin-sulbactam. Variable activities of tetracyclines, trimethoprim-sulfamethoxazole, and ampicillin toward *C. jejuni* strains, similar to that seen for aerotolerant *Campylobacter* organisms, have been reported (7, 11, 14, 16, 22, 26, 29, 42, 46, 54). However, other *Campylobacter* species (*C. fetus* subsp. *fetus* [30], *C. cinaedi*, and *C. fennelliae* [15]) are generally considered susceptible to these antimicrobial agents. Isolates of *C. lari* are generally susceptible to tetracycline, but like aerotolerant *Campylobacter* organisms, demonstrate variable susceptibility toward trimethoprim-sulfamethoxazole (31, 43, 47). Increased levels of resistance of aerotolerant *Campylobacter* organisms to ampicillin (range, 4 to 32) were
also noted, which is not typical of levels obtained when testing other Campylobacter species. The activity of ampicillin was not enhanced significantly by the addition of sulbactam for the study organisms; similar results have been noted for C. jejuni and C. fetus subsp. fetus (14, 51).

An additional goal of this study was to discriminate between strains belonging to C. cyaerophila DNA hybridization group 1B and C. butzleri. These groups are currently difficult to distinguish using phenotypic tests (24). However, overlapping MICs were noted between the hybridization groups. The MICs of strains D2883 and D2884, which genetically belong to DNA hybridization group 1B but phenotypically more closely resemble hybridization group 1A, were similar to those of isolates of C. cyaerophila DNA hybridization group 1A. In addition, differences were not noted among the three subsets of C. butzleri strains, including U.S. animal isolates (primarily of macaque origin), strains isolated in Thailand, and U.S. isolates submitted to the Centers for Disease Control for identification. This is in contrast to reports showing that strains of C. jejuni and C. coli isolated from locations outside the United States demonstrate higher MICs to antimicrobial agents such as erythromycin, chloramphenicol, and tetracycline, that are more readily available in these countries than they are in the United States (29, 36, 48).

Previous investigations in our laboratory noted that aerotolerant Campylobacter organisms generally did not grow on a selective medium (Campy-BAP) commonly used in primary isolation of Campylobacter organisms (24). To determine which antimicrobial agents might be responsible for this, we included 10 additional antimicrobial agents at concentrations commonly used in commercial media. Aerotolerant Campylobacter isolates were typically susceptible to colistin, polymyxin B, and rifampin. These antimicrobial agents are included in commercially available media based on several formulations, namely, those of Skirrow (containing trimethoprim, polymyxin B, and vancomycin) (44), Butzer and Skirrow (containing bacitracin, cycloheximide, colistin, cefazolin, and novobiocin) (9), Blaser et al. (Campy-BAP; containing vancomycin, polymyxin B, trimethoprim, amphotericin B, and cephalothin) (4), Bolton and Robertson (Preston medium containing polymyxin B, rifampin, trimethoprim, and cycloheximide) (6) and modification thereof (5), and Fennell and colleagues’ modification of Skirrow’s formulation by the addition of amphotericin B (13). These antimicrobial agents are not present in commercially available formulations of CVA (containing cefoperazone, vancomycin, and amphotericin B) (38), Karmali (containing cefoperazone, vancomycin, and cycloheximide) (23), or a modified Preston formula (CCDA; containing cefoperazone) (28). Some formulations of selective media for Helicobacter pylori may also be suitable for isolation of aerotolerant Campylobacter organisms; however, each formulation must be carefully evaluated for the presence of colistin, polymyxin B, and rifampin. An additional and important consideration when isolating these organisms is that most aerotolerant Campylobacter strains will not grow at 42°C (24). Lack of growth on certain selective media has been reported for other Campylobacter species, such as C. upsaliensis (37, 45), C. fennelliae, and C. cinaedi (13), in addition to a few strains of C. jejuni and C. coli (21, 27, 34, 35). An increasing recognition of the clinical significance of these organisms, in

### Table 2. Susceptibilities to agents commonly used in selective media

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Conc tested</th>
<th>C. butzleri (n = 64)</th>
<th>DNA hybridization group 1A (n = 6)</th>
<th>DNA hybridization group 1B (n = 6)</th>
<th>D2883*</th>
<th>D2884*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>2 µg/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Bacitracin</td>
<td>25 U/ml</td>
<td>0 (0)</td>
<td>2 (33)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>15 µg/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Colistin</td>
<td>10 U/ml</td>
<td>60 (94)</td>
<td>6 (100)</td>
<td>5 (83)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>50 µg/ml</td>
<td>0 (0)</td>
<td>1 (17)</td>
<td>0 (0)</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>5 U/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>5 U/ml</td>
<td>59 (92)</td>
<td>6 (100)</td>
<td>5 (83)</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>2.5 U/ml</td>
<td>44 (69)</td>
<td>6 (100)</td>
<td>2 (33)</td>
<td>S</td>
<td>S</td>
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<tr>
<td>Rifampin</td>
<td>10 U/ml</td>
<td>38 (59)</td>
<td>3 (50)</td>
<td>1 (17)</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>5 µg/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>10 µg/ml</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td>R</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>4 µg/ml</td>
<td>30 (47)</td>
<td>6 (100)</td>
<td>0 (0)</td>
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</tr>
<tr>
<td></td>
<td>32 µg/ml</td>
<td>59 (92)</td>
<td>6 (100)</td>
<td>1 (17)</td>
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<td>S</td>
</tr>
<tr>
<td></td>
<td>64 µg/ml</td>
<td>61 (95)</td>
<td>6 (100)</td>
<td>3 (50)</td>
<td>S</td>
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</tbody>
</table>

* Strains D2883 and D2884 belong to DNA hybridization group 1B; however, they phenotypically resemble C. cyaerophila DNA hybridization group 1A. R, resistant; S, susceptible.
addition to aerotolerant *Campylobacter* strains, should prompt investigation of alternative media or additional procedures for primary isolation of *Campylobacter* species.

In conclusion, human and most animal isolates of aerotolerant *Campylobacter* organisms were generally not susceptible to antimicrobial agents commonly used to treat other diarrheagenic campylobacters, e.g., erythromycin, other macrolide antibiotics, cephalosporins, amoxicillin and ampicillin-sulfactam, clindamycin, chloramphenicol, and trimethoprim-sulfadiazinazole. In vitro laboratory data suggest that these antimicrobial agents may not be appropriate for treatment of diarrheal illness associated with aerotolerant *Campylobacter* organisms and that minocycline, the quinolones, or an aminoglycoside should be considered. Additionally, isolates are susceptible to antimicrobial agents used in commercially available *Campylobacter* media (colistin, polymyxin B, and rifampin). Laboratories interested in isolating aerotolerant *Campylobacter* organisms must modify existing procedures, and laboratories that isolate aerotolerant *Campylobacter* organisms from diarrheal stools need to be aware of the unusual antimicrobial susceptibility patterns.

**REFERENCES**