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In Vitro Susceptibilities of *Campylobacter*-Like Organisms to Twenty Antimicrobial Agents

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We determined MICs of 20 antimicrobial agents for 50 representative strains of four subgroups of *Campylobacter*-like organisms (CLOs) by agar dilution. Ampicillin, gentamicin, doxycycline, tetracycline, ceftriaxone, rifampin, spectinomycin, nalidixic acid, and chloramphenicol were active against all strains of CLOs. Most CLO strains (83%) were inhibited by 4 μg of sulfamethoxazole per ml and by 8 μg of trimethoprim-sulfamethoxazole per ml. Of type 1 strains, 28% were resistant to 8 μg of erythromycin per ml. In addition, cross resistance between erythromycin and clindamycin was always present. Type 1 strains exhibited a broad distribution of MICs of metronidazole and streptomycin, whereas all type 2 strains were uniformly susceptible to metronidazole and resistant to streptomycin. Unlike type 1 and 3 strains, type 2 CLOs were susceptible to cephalothin and penicillin G and highly resistant to streptomycin. The type 3 strain was uniquely resistant to cefazolin. The majority of strains were not inhibited by cefoperazone; and all were resistant to trimethoprim. In contrast to *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus*, all CLOs tested were susceptible to 0.5 μg of rifampin per ml.

Increased awareness of the role of *Campylobacter* spp. in human disease and the development of improved selective media have resulted in recent isolation of several novel *Campylobacter* spp. from humans (7, 8, 10, 13, 14, 16–18). Among these, we isolated *Campylobacter*-like organisms (CLOs) from symptomatic and asymptomatic homosexual men being evaluated for enteritis, proctitis, and proctocolitis in a sexually transmitted diseases clinic. These organisms were isolated by using a selective medium containing vancomycin, polymyxin B, trimethoprim, and amphotericin B supplemented with 10% sheep blood (2) after incubation at 37°C in a microaerophilic environment. The classification of these CLOs within the genus *Campylobacter* and their phenotypic characterization into three subgroups were recently described by Fennell et al. (8). Further studies revealed four genetically distinct CLO subgroups (types 1A, 1B, 2, and 3), two of which (types 1A and 1B) were phenotypically identical (21a). The spectrum of diseases associated with CLO infection includes asymptomatic gastrointestinal colonization (17), proctocolitis (17), and bacteremia (18), but little is known about the susceptibility of these organisms to antimicrobial agents. In this study, we determined the MICs of 20 antimicrobial agents for 50 strains of CLOs.

MATERIALS AND METHODS

Bacterial strains. We studied 50 strains of CLOs isolated from homosexual men, including 39 type 1A strains, 4 type 1B strains, 6 type 2 strains, and 1 type 3 strain. Of the 50 strains, 48 were isolated from rectal swabs of symptomatic and asymptomatic patients seen in the Harborview Medical Center Sexually Transmitted Diseases Clinic, Seattle, Wash. Two CLO type 1A strains were blood culture isolates from immunocompromised homosexual patients provided by Roy L. Hopfer, Houston, Tex. Reference strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were obtained from the American Type Culture Collection.

Isolates were preserved at −70°C in 1:1 proportions of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and inactivated horse serum. Suspensions were thawed as needed, inoculated onto brucella agar (Difco Laboratories, Detroit, Mich.) supplemented with 10% defibrinated sheep blood, and incubated for 48 h at 37°C in GasPak jars (BBL) containing H_2–CO_2-generating GasPak envelopes (BBL) with the catalyst removed.

Antimicrobial agents. The following antimicrobial agents were tested: ampicillin trihydrate (Bristol Laboratories, Syracuse, N.Y.), cephalothin sodium, cefazolin sodium, streptomycin sulfate (Eli Lilly & Co., Indianapolis, Ind.), tetracycline hydrochloride, doxycycline hyclate, cefoperazone sodium (Pfizer Inc., New York, N.Y.), clindamycin hydrochloride, erythromycin, spectinomycin hydrochloride (The Upjohn Co., Kalamazoo, Mich.), chloramphenicol (Parke, Davis & Co., Detroit, Mich.), sulfamethoxazole, trimethoprim lactate (Burroughs Wellcome Co., Research Triangle Park, N.C.), penicillin G (E. R. Squibb & Sons, Princeton, N.J.), rifampin (Merrell Dow Pharmaceuticals Inc., Cincinnati, Ohio), nalidixic acid (United States Biochemical Corp., Cleveland, Ohio), gentamicin sulfate (Schering Corp., Bloomfield, N.J.), metronidazole (G. D. Searle & Co., San Juan, P.R.), and ceftriaxone sodium (Hoffman-La Roche, Nutley, N.J.).

Susceptibility tests. Susceptibility testing was performed by a modified agar dilution method (27). Diagnostic sensitivity test agar (Oxoid Ltd., London, England) (pH 7.4) with 10% lysed defibrinated horse blood was used for testing sulfamethoxazole and trimethoprim, whereas tests against other antimicrobial agents were performed with Mueller-Hinton agar (Difco; pH 7.2) supplemented with 10% defibrinated sheep blood. Log_2 dilutions of each antibiotic were incorporated into the appropriate test medium and used within 24 h of preparation. Trimethoprim-sulfamethoxazole was tested at a ratio of 1:19.

Cultures (48 h) of CLOs were suspended in Trypticase soy broth to match the turbidity of a 0.5 McFarland standard and then diluted 1:20 in 0.85% saline (pH 7.0). This suspension yielded 10^6 to 10^7 CFU/ml as determined by colony count. Samples (1 to 2 μl) of the diluted suspensions were inocu-

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TABLE 1. Susceptibilities of CLO type 1A and 1B[a] strains to 20 antimicrobial agents

Antibiotic	No. of strains tested	MIC (μg/ml) Range	50%[b]	90%[b]
Ampicillin	43	1–8	4	8
Penicillin G	38	2–16	8	16
Gentamicin	42	0.12–1	0.5	1
Streptomycin	42	1–>256	4	>256
Spectinomycin	43	2–16	4	4
Doxycycline	39	≤0.06–1	0.25	0.5
Tetracycline	43	0.12–4	0.5	2
Chloramphenicol	42	0.12–2	0.5	1
Nalidixic acid	41	2–16	8	16
Rifampin	41	0.03–0.5	0.25	0.25
Clindamycin	43	≤0.06–64	0.12	16
Erythromycin	43	≤0.06–>128	≤0.06	>128
Metronidazole	42	≤0.06–128	1	32
Sulfamethoxazole	41	0.5–64	2	32
Trimethoprim	41	64–>512	256	>512
Trimethoprim-sulfamethoxazole	41	1–64	4	64
Cephalothin	42	16–64	32	64
Cefoperazone	43	16–>128	64	>128
Ceftriaxone	37	1–8	4	8
Cefazolin	40	1–16	4	16

[a] MICs for CLO types 1A and 1B were combined because of similar values.
[b] MIC required to inhibit 50 or 90% of strains as indicated.

lated with a multipoint replicator as described by Steers et al. (20). Plates containing no antibiotics were inoculated to determine viability and quantitation of growth. Inoculated plates were incubated at 37°C for 48 h in a microaerophilic environment as previously described. The concentration which inhibited at least 80% of growth was interpreted as the MIC of trimethoprim, sulfamethoxazole, and trimethoprim-sulfamethoxazole. For the other antimicrobial agents tested, the concentration at which there was complete inhibition of growth or the presence of a barely visible haze was determined as the MIC.

Reference strains of *S. aureus* and *E. coli* were included in every test to monitor the antibiotic potency in the medium. Reproducibility of results was determined by testing most strains several times with different lots of antibiotic-containing media. MICs for each strain which were within the "acceptable range" of 1 $\log_2$ dilution of previous values were accepted, and the higher of the two values was chosen as the MIC.

RESULTS

Antimicrobial agents active against all 50 CLO strains tested included ampicillin, gentamicin, spectinomycin, doxycycline, tetracycline, chloramphenicol, nalidixic acid, rifampin, and ceftriaxone (Tables 1 and 2). The majority of strains (83%) were susceptible to sulfamethoxazole (<4 μg/ml) and trimethoprim-sulfamethoxazole (<8 μg/ml), whereas 76% were resistant to 64 μg of cefoperazone per ml. All strains were resistant to trimethoprim.

CLO type 2 strains were highly resistant to streptomycin (Table 2) and uniformly susceptible to clindamycin, erythromycin, and metronidazole, whereas the type 1 strains exhibited a broad range of MICs of these antimicrobial agents

TABLE 2. MICs for CLO type 2 and 3 strains

Antibiotic	MIC (μg/ml) for CLO strain: Type 2						Type 3
	74	231	441	528	797	897	37
Ampicillin	1	1	2	2	1	2	2
Penicillin G	2	1	ND[a]	0.5	0.5	0.12	4
Gentamicin	0.5	0.5	0.12	0.25	0.25	0.25	0.5
Streptomycin	>256	>256	>256	>256	>256	>256	1
Spectinomycin	4	2	2	4	2	4	8
Doxycycline	0.25	0.25	0.25	ND	0.12	0.5	0.25
Tetracycline	0.5	1	1	0.12	0.25	1	2
Chloramphenicol	0.5	0.5	ND	0.5	1	4	8
Nalidixic acid	16	8	8	4	8	8	32
Rifampin	0.06	0.12	0.06	0.06	0.06	0.06	0.12
Clindamycin	0.25	0.25	0.5	0.12	0.25	0.5	0.25
Erythromycin	0.25	0.25	0.5	0.5	0.12	0.5	≥0.06
Metronidazole	0.12	0.12	0.12	0.12	≤0.06	0.12	16.0
Sulfamethoxazole	2	2	1	2	1	2	4
Trimethoprim	128	128	128	256	256	128	512
Trimethoprim-sulfamethoxazole	2	2	2	2	1	4	4
Cephalothin	4	4	8	8	4	8	>128
Cefoperazone	16	16	ND	128	32	128	>128
Ceftriaxone	0.5	0.5	0.5	0.5	0.5	0.5	4
Cefazolin	1	ND	ND	≤0.06	2	4	128

[a] ND, Not done.

(Table 1). Of type 1 strains, 12 (28%) were resistant to erythromycin, and all of these erythromycin-resistant strains showed cross resistance to clindamycin. Generally, for all agents active against type 2 strains, the range of MICs was lower than the range of MICs obtained for type 1 strains. The single CLO type 3 strain was highly resistant to cephalothin and cefazolin.

DISCUSSION

In general, the MICs of most of the antimicrobial agents tested against CLOs were within the ranges characteristic of *Campylobacter jejuni* (12, 15, 19, 24, 26) and *Campylobacter fetus* subsp. *fetus* (4, 6). However, there were several differences which distinguished CLOs from these other species. CLOs differed from *C. jejuni* and *C. fetus* subsp. *fetus* by their susceptibility to 0.5 μg of rifampin per ml (1, 6, 12, 19). Like most *C. jejuni* strains, CLOs could be distinguished from *C. fetus* subsp. *fetus* by their susceptibility to <40 μg of nalidixic acid per ml (12, 24, 25). CLO type 1 and 2 strains were susceptible to cefazolin but strains of *C. jejuni* that are susceptible to this antimicrobial agent have not been reported (11, 15).

Recently described formulations designed for *C. jejuni* isolation contain at least one of the following antimicrobial agents in concentrations (milligrams per liter) inhibitory to CLOs: cephalothin, 15 (2); cefazolin, 15 (5); rifampin, 10 (9); and cefoperazone, 20 (L. B. Reller, S. Mirrett, and L. G. Reimer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C274, p. 357). The selective isolation medium we used is a modification of Skirrow medium. It contains vancomycin (10 mg/liter), polymyxin B (2,500 IU/liter), amphotericin B (2 mg/liter), and trimethoprim (5 mg/liter) (2) and supports the growth of all the CLO subgroups.

Of the CLO strains tested, 24% were resistant to erythromycin and clindamycin. This proportion is considerably higher than that previously reported for *C. jejuni* (erythromycin resistance of 0 to 12.6% of strains) (3, 12, 15, 26). Erythromycin resistance of CLOs was always accompanied by clindamycin resistance. Although clindamycin and erythromycin are chemically distinct, they utilize the same mechanism for inhibition of protein synthesis and the same receptor site on the bacterial ribosome. Bacteria lacking this receptor site are not susceptible to the action of either antimicrobial agent. In contrast to numerous reports of tetracycline resistance among *C. jejuni* (3, 12, 15, 21, 22, 24), all CLOs were susceptible to tetracycline and to doxycycline. Based on these data, erythromycin, generally considered the drug of choice for treatment of campylobacter gastrointestinal infections, may be less appropriate for CLO therapy than tetracycline or doxycycline. However, clinical data are needed to substantiate these in vitro results.

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