ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1980, p. 118-121
0066-4804/80/07-0118/04$02.00/0

Vol. 18, No. 1

Bacteriostatic and Bactericidal Activities of 24 Antimicrobial Agents Against *Campylobacter fetus* subsp. *jejuni*

R. VANHOOF,* B. GORDTS, R. DIERICKX, H. COIGNAU, AND J. P. BUTZLER

Department of Microbiology, St. Pieters Hospital, Free University of Brussels, 1000 Brussels, Belgium

The bacteriostatic and bactericidal activities of 24 antimicrobial agents were tested with the Dynatech MIC 2000 system against 86 strains of *Campylobacter fetus* subsp. *jejuni* from human sources. The penicillins (penicillin G, ampicillin, amoxycillin, carbenicillin) had poor activity. Ampicillin and amoxycillin were equally active. Cefotaxime revealed a rather good activity. Erythromycin, gentamicin, tobramycin, amikacin, and furazolidone were the most active compounds. Two strains (2.3%) were resistant to erythromycin. One strain (1.2%) was completely resistant to tobramycin. The tetracyclines (tetracyline, doxycycline, minocycline) were generally effective, but 8% of the strains were totally resistant to them. Minocycline was the most active. Chloramphenicol, thiamphenicol, and clindamycin had good activity. The bacteriostatic and bactericidal distributions for colistin, nalidixic acid, and metronidazole were broad.

Since King (8) identified campylobacters as a possible cause of enteritis in humans, these microorganisms have become important pathogens, especially since 1972 when workers in Brussels (5) facilitated the isolation of the organisms by use of a selective technique. Since that time *Campylobacter fetus* subsp. *jejuni (C. jejuni)* became a frequently isolated enteropathogenic microorganism (2, 7, 13) with a worldwide distribution (6, 9, 12). It has also been associated with other pathologies (4, 11, 19).

The seriously ill and septicemic patient should be treated, but until now there has been little information to guide the therapy. The aim of this study was to determine the in vitro activity of various antibiotics, as well as their degree of bactericidal action on campylobacters.

MATERIALS AND METHODS

Bacterial strains. Eighty-six strains of *C. jejuni* isolated from human stools (5) were used in this study. The strains were preserved in fluid thioglycolate agar medium (no. 0256-01, Difco Laboratories, Detroit, Mich.) and stored in liquid nitrogen or kept in a freezer at −70°C. When required, the suspensions were thawed and inoculated onto petri dishes containing fluid thioglycolate agar medium with 1.5% agar and 10% defibrinated sheep blood (Institute Pasteur, Brussels) and (per milliliter) 25 IU of bacitracin (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.005 mg of novobiocin (The Upjohn Co., Kalamazoo, Mich.), and 0.05 mg of cycloheximide (The Upjohn Co.).

Inoculum. The inoculated plates were incubated at 37°C in a 10% CO_2 atmosphere for 2 days. The colonies from the plates were then suspended in 10 ml of fluid thioglycolate medium and incubated for 24 h in 10% CO_2. The turbidity of these overnight cultures was adjusted to match a McFarland no. 1 standard. These cultures were then inoculated into microtiter plates containing 0.1 ml of broth so that the final inoculum contained approximately 3×10^6 colony-forming units per ml. The CO_2 was controlled with a Portomatic CO_2 controller (3056/3062, Forma Scientific Inc.).

Antibiotic susceptibility tests. A Cooke Dynatech 2000 dispensor and inoculator, with a 1-μl amount of inoculum, were used for the determination of the minimal inhibitory concentration (MIC) in fluid medium.

The microtiter plates (Dynatech MIC plates MA 1501/N) were filled with 0.1-ml samples of the antibiotic solutions prepared in Mueller-Hinton broth (MHB) (40702, Bio-Mérieux), except that for sulfamethoxazole, trimethoprim, and co-trimoxazole, the medium was MHB with 5% hemolyzed horse blood (Institute Pasteur, Brussels). The microtiter plates were incubated in a 10% CO_2 atmosphere at 37°C for 48 h. In every microtiter plate, one row without antibiotics served as a control. For each antibiotic, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) with known antibiotic susceptibility patterns were tested at the same time. The trays were examined on a Cooke-Dynatech viewing box. MICs were recorded as the lowest concentrations inhibiting visible growth.

To measure the minimal bactericidal concentrations (MBC), we plated the MIC cultures on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.; 11438) with 5% lysed horse blood and incubated them for 24 h at 37°C in a 10% CO_2 atmosphere. A reduction of at least 90% of the colonies, compared with the culture of the initial inoculum of the strain, was regarded as evidence of bactericidal activity.

Antibiotics. The following antimicrobial agents were tested: ampicillin, amoxycillin, and carbenicillin (Beecham Laboratory), benzylpenicillin (Continental Pharma), cefazolin, moxalactam, and tobramycin (Eli

Lilly & Co.), cefotaxime (Hoechst), erythromycin (Abbott Laboratories), thiamphenicol (Zambon), chloramphenicol (Lepetit), clindamycin (Upjohn), gentamicin (Schering Corp.), amikacin (Bristol Laboratories), tetracycline (CERTA), doxycycline (Pfizer, Inc.), minocycline (Lederle Laboratories), colistin (Bellon Laboratories), nalidixic acid (Winthrop), furazolidone (Norwich, Benelux), metronidazole (Specia), sulfamethoxazole, trimethoprim (Roche S.A.), and co-trimoxazole, the 20:1 combination of sulfamethoxazole and trimethoprim.

Statistical analysis. We calculated the geometric mean MIC and MBC for every antimicrobial agent.

For comparison of the difference between MIC and MBC, we used a one-sided t-test for related samples. The paired comparison of MIC or MBC values was done by a two-sided t-test for related samples.

RESULTS

MICs. The range of MICs, the geometric mean MIC, and the concentrations of the various drugs needed for inhibition of 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) are listed in Table 1.

In general, the penicillins showed low activity towards campylobacter strains. Although ampicillin and amoxycillin had a rather low geometric mean MIC, the MIC_{90}s of these penicillins were 12.5 μg or more per ml. In the group of cephalosporins, only cefotaxime showed activity against the strains tested. The MIC_{90} of 6.25 μg/ml is achievable in serum. Erythromycin, clindamycin, the aminoglycosides, the tetracyclines, and furazolidone were the most active compounds of the 24 antimicrobial agents.

Only two strains showed a high resistance to erythromycin. Clindamycin, with a geometric mean MIC of 0.13 μg/ml and an MIC_{90} of 0.39 μg/ml, is one of the most active compounds. Gentamicin is the most potent aminoglycosidic antibiotic. All strains were fully susceptible to gentamicin, and it differed significantly from tobramycin and amikacin ($P < 0.001$). One strain was completely resistant to tobramycin (MIC = 25 μg/ml).

The tetracyclines had rather good activity, but 8.1% of the strains were completely resistant (MIC > 6.25 μg/ml). Minocycline was the most active tetracycline compound. The geometric mean MIC value for furazolidone was 0.09 μg/ml.

Although all the strains had the biochemical characteristics of *C. jejuni*, the MIC for 28 strains (32.6%) was > 6.25 μg of nalidixic acid per ml. Metronidazole had no good activity.

Chloramphenicol and thiamphenicol had MIC_{90}s of 3.12 and 6.25 μg/ml, respectively ($0.7 < P < 0.8$). We found the activity of colistin controversial because the strains were initially isolated on a selective medium containing colistin.

All the strains were resistant to trimethoprim. Sulfamethoxazole and co-trimoxazole were only moderately active: 38 and 58% of the strains

TABLE 1. *Susceptibility of C. fetus subsp. jejuni to antimicrobial agents*

Drug	MIC (μg/ml of medium)			
	Range	MIC_{50}	MIC_{90}	Geometric mean
Penicillin G	≤0.048–50	6.25	12.5	4.60
Ampicillin	≤0.048–50	3.12	12.5	2.15
Amoxycillin	≤0.048–>50	3.12	12.5	2.53
Carbenicillin	≤0.048–>50	6.25	25	6.35
Cefazolin	0.097–>50	25	50	12.90
Cefotaxime	≤0.048–50	3.12	6.25	2.00
Moxalactam	0.195–>50	12.5	50	10.55
Erythromycin	≤0.048–>50	0.195	0.78	0.24
Thiamphenicol	≤0.048–>50	1.56	6.25	1.21
Chloramphenicol	≤0.048–12.5	1.56	3.12	1.26
Clindamycin	≤0.048–12.5	0.097	0.39	0.13
Gentamicin	≤0.048–0.78	0.097	0.195	0.14
Tobramycin	≤0.048–25	0.39	1.56	0.48
Amikacin	≤0.048–3.12	0.39	0.78	0.39
Tetracycline	0.097–>50	0.195	1.56	0.36
Doxycycline	≤0.048–>50	0.097	1.56	0.22
Minocycline	≤0.048–>50	≤0.048	0.39	0.12
Colistin	≤0.048–50	0.78	3.12	0.96
Nalidixic acid	0.195–>50	6.25	50	6.61
Furazolidone	≤0.048–0.78	0.097	0.195	0.09
Metronidazole	0.097–>50	12.5	50	7.58
Sulfamethoxazole	≤0.97–>1,000	15.6	125	18.94
Trimethoprim	12.5–>200	200	>200	198.36
Co-trimoxazole	≤0.97–>1,000	31.2	125	31.47

TABLE 2. *Susceptibility of C. fetus subsp. jejuni to antimicrobial agents*

Drug	MBC (μg/ml of medium)			
	Range	MBC_{50}	MBC_{90}	Geometric mean
Penicillin G	0.097->50	12.5	50	8.56
Ampicillin	0.097->50	3.12	25	3.79
Amoxycillin	0.097->50	3.12	25	3.58
Carbenicillin	≤0.048->50	12.5	50	10.81
Cefazolin	0.097->50	50	50	27.44
Cefotaxime	0.097->50	3.12	25	3.79
Moxalactam	0.78->50	25	>50	18.40
Erythromycin	≤0.048->50	0.39	1.56	0.48
Thiamphenicol	≤0.048->50	1.56	25	2.41
Chloramphenicol	≤0.048->50	1.56	25	2.61
Clindamycin	≤0.048->50	0.195	12.5	0.32
Gentamicin	≤0.048->50	0.195	3.12	0.29
Tobramycin	≤0.048->50	0.78	6.25	0.92
Amikacin	≤0.048->50	0.78	3.12	0.87
Tetracycline	0.097->50	0.39	6.25	0.61
Doxycycline	≤0.048->50	0.195	6.25	0.49
Minocycline	≤0.048->50	0.195	1.56	0.28
Colistin	0.097->50	1.56	12.5	2.12
Nalidixic acid	0.39->50	6.25	>50	9.42
Furazolidone	≤0.048-12.5	0.097	1.56	0.16
Metronidazole	0.39->50	25	50	13.22
Sulfamethoxazole	1.95->1,000	62.5	>1,000	73.97
Trimethoprim	25->200	>200	>200	269.47
Co-trimoxazole	1.95->1,000	125	>1,000	110.74

showed resistance to the two compounds, respectively.

MBCs. The range of MBCs, the geometric mean MBC, and the concentrations of the various drugs needed to kill 50 and 90% (MBC_{50} and MBC_{90}) of strains are listed in Table 2. In general, the MBCs differed significantly from the MICs ($P < 0.001$); this means that the antibiotics exhibited a bacteriostatic activity towards the campylobacters. The amount of drug necessary to increase the inhibitory concentration to a bactericidal one varied from 36% (trimethoprim) to 252% (co-trimoxazole).

In the group of the beta-lactams, ampicillin, amoxycillin, and cefotaxime seemed to have almost the same killing properties. The geometric mean MBCs of these three compounds were, respectively, 3.79, 3.58, and 3.79 μg/ml. The three antibiotics did not differ significantly ($P > 0.3$). More than 50% of the strains were killed by concentrations of these antibiotics easily achievable in the blood. For erythromycin, gentamicin, amikacin, minocycline, and furazolidone, the MBC_{90}s might have a clinical importance. With the exception of furazolidone, the MBC_{90}s are attainable in the blood.

DISCUSSION

The results of our in vitro study on the bacteriostatic and bactericidal activities of 24 antimicrobial agents on 86 strains of *C. jejuni* were in general agreement with those obtained by other investigators (1, 3, 16, 17). It is accepted that beta-lactam antibiotics are not very active on campylobacters. In this study, we have found a rather good activity of cefotaxime, a new semisynthetic parenteral cephalosporin. This has also been reported previously (15). A total of 90% of the strains were inhibited by 6.25 μg/ml, and this concentration killed 80% of the strains. A concentration of 6.25 μg/ml is easily achievable in the blood with a normal dose (10). Clinical results obtained with ampicillin are very variable, and this is reflected by our in vitro results. Good clinical results have been obtained with erythromycin. Nevertheless, erythromycin-resistant strains have been reported. Vanhoof et al. (16) judged 8.4% of their strains to be resistant, and Walder and Forsgren (17, 18) reported the same proportion. However, Telfer-Brunton et al. (14) in Britain reported a figure of 0.5%. In our study erythromycin exhibited good bacteriostatic and bactericidal activities, and only two strains (2.3%) were judged as resistant. The reasons for the difference in frequency of erythromycin resistance are not known.

The same problem of resistance is encountered in the group of the tetracyclines. These compounds have good in vitro bacteriostatic activities, but seven strains (8.1%) showed a high-level resistance to the three compounds tested. In a previous study, we found a proportion of 5% (16). Clindamycin and the aminoglycosidic antibiotics were the most active parenteral antibi-

otics. Furthermore, gentamicin was the most active aminoglycoside. Furazolidone exhibits very good bacteriostatic and bactericidal activities, but the clinical use of this compound is limited since it exerts only a contact effect in the gut.

ACKNOWLEDGMENT

We are very indebted to L. Kaufman, Department of Statistics and Operations Research, Free University, Brussels, for his statistical advice and for carrying out the statistical analysis.

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