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Antimicrobial Susceptibilities of Anaerobic Bacteria: Recent Clinical Isolates

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Minimal inhibitory concentrations of clindamycin, minocycline, metronidazole, penicillin, and carbenicillin were determined by agar dilution against 150 recent clinical isolates of anaerobic bacteria. Ninety-nine percent of *Bacteroides fragilis* and all *B. melaninogenicus*, *Clostridium perfringens*, and *Fusobacterium* were inhibited by clindamycin at 3.1 μg/ml. Only 58% of other clostridial species were inhibited by this concentration of clindamycin. Minocycline at 3.1 μg/ml inhibited 72% of *C. perfringens*, 81% of other *Clostridium* species, and 66, 75, and 100% of *B. fragilis*, *B. melaninogenicus*, and *Fusobacterium*, respectively. Metronidazole at 12.5 μg/ml inhibited all bacteria tested. *B. fragilis* was resistant to both penicillin and carbenicillin at 6.2 μg/ml. Concentrations of 25 μg/ml for penicillin and 100 μg/ml for carbenicillin were needed to inhibit more than 90% of *B. fragilis*. Organisms other than *B. fragilis* were moderately or extremely susceptible to the penicillins.

Because of the relatively long in vitro generation times and the fastidious nature of anaerobic bacteria, rapid determination of antimicrobial susceptibilities is not feasible. Therefore, it is necessary to monitor the specific susceptibility patterns of recent clinical isolates for possible changes in resistance, particularly to widely used drugs. A survey of the in vitro antimicrobial susceptibilities of anaerobic bacteria isolated during 1971 was reported from this laboratory by Martin et al. (17).

For the present in vitro study, clindamycin was chosen because it has been recommended by several investigators as one of the drugs of choice for treatment of anaerobic infections (2, 3, 7, 10, 11, 16, 27). Minocycline was included because it is a derivative of tetracycline, the former drug of choice against *Bacteroides fragilis* infection (13). Recent encouraging reports on the effectiveness of metronidazole against anaerobes (19, 26, 29) prompted us to investigate the activity of this antiparasitic drug. Penicillin and carbenicillin were evaluated to determine the possibility of using high doses of penicillin or its derivatives as components of the initial antimicrobial therapy of infections when the cause is unknown but may include anaerobic bacteria.

MATERIALS AND METHODS

The 150 anaerobic strains were isolated from a variety of clinical specimens submitted to the Section of Clinical Microbiology, Mayo Clinic, between October 1973 and February 1974. Isolation and identification procedures were performed essentially according to the recommendations of Dowell and Hawkins (8). Gas-liquid chromatographic analysis of fermentation acids and the interpretation of these data were as described in the *Anaerobic Laboratory Manual* of the Virginia Polytechnic Institute (12). To keep isolated strains until susceptibility testing was performed, equal volumes of a thioglycolate broth (enriched with rabbit serum) culture and whole defibrinated sheep blood were mixed, flash-frozen in an alcohol-Dry Ice bath, and stored at −60 C. All anaerobic incubation in this study was in the GasPak system (BBL) at 37 C.

Antimicrobial susceptibility testing was performed by the agar dilution method essentially as described by Sutter and Washington (25). The antimicrobial agents, in appropriate dilutions in sterile distilled water, were incorporated into a brain heart infusion agar base with added 5% whole defibrinated sheep blood and vitamin K_1 (menadione, 0.5 μg/ml). The concentration of metronidazole was calculated on a dry weight basis; the remaining drugs were diluted on the basis of the activity specified by the manufacturer.

For the inocula, cultures of anaerobes were grown for 48 h in thioglycolate-rabbit serum broth and diluted in plain thioglycolate broth to match a McFarland no. 1 barium sulfate standard. Inocula were applied to the plates by means of the replicating device of Steers et al. (22) so that approximately 10^5 colony-forming units were deposited on the surface of the agar. Control strains with known minimal inhibitory concentration (MIC) values, as well as control plates for anaerobic and aerobic growth and contamination, were included in each test series. After 48 h of anaerobic incubation at 37 C, the MIC was deter-

mined as the lowest concentration of drug permitting no growth, only one or two discrete colonies, or a barely visible fine haze.

RESULTS

All but one of the 70 *B. fragilis* strains tested, all strains of *Clostridium perfringens*, and all *Fusobacterium* species were susceptible to clindamycin at 3.1 μg/ml or less (Table 1). All other *Bacteroides* species were inhibited by this antibiotic at 0.4 μg/ml or less. However, only 58% of species of *Clostridium* other than *C. perfringens* were inhibited by clindamycin at 3.1 μg/ml. Table 2 lists these species of *Clostridium* with their individual MIC values.

Minocycline at a concentration of 3.1 μg/ml inhibited 66 and 75% of strains of *B. fragilis* and *B. melaninogenicus*, respectively (Table 3). Within the genus *Clostridium*, 72% of the strains of *C. perfringens* and 81% of the other species were susceptible to 0.4 μg/ml or less. All seven strains of *Fusobacterium* tested were inhibited by minocycline at 0.2 μg/ml.

At a concentration of 12.5 μg/ml, metronidazole inhibited all strains used in this study (Table 4).

At 6.2 μg/ml, penicillin was effective against 96 and 87% of the strains of *C. perfringens* and other clostridial species, respectively, as well as against all *Fusobacterium* strains tested (Table 5). However, only 7% of strains of *B. fragilis* were inhibited by this concentration. Penicillin was more effective against *B. melaninogenicus* and the two other *Bacteroides* species tested here (*B. pneumosintes* and *B. clostridiiformis*). Carbenicillin at 100 μg/ml inhibited 99% of the strains of *B. fragilis* (Table 6). The other *Bacteroidaceae* were inhibited by 12.5 μg/ml and in most instances by considerably lower concentrations. All strains of *C. perfringens* were inhibited by 12.5 μg/ml and 96% were inhibited by 0.8 μg/ml. Other species of *Clostridium* were less susceptible to carbenicillin.

DISCUSSION

Recent in vitro and in vivo studies have demonstrated the activity of clindamycin against anaerobic bacteria, particularly *B. fra-*

TABLE 1. *Minimal inhibitory concentrations of clindamycin*

Organism	Strains tested	Cumulative percentages at increasing concn (μg/ml)									
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	>25
Bacteroides fragilis	70	51	69	83	87	93	99	100			
B. melaninogenicus	20	95		100							
Bacteroides sp.	2	100									
Clostridium perfringens	25	44		48	52	88	100				
Clostridium sp.	26	23	27	38		54	58	77	88	92	100
Fusobacterium sp.	7	57				100					

TABLE 2. *Susceptibility of Clostridium species to clindamycin*

Organism	Minimal inhibitory concn (μg/ml)									
	0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	>25
C. aminovalericum					X					
C. barati					X					
C. barkeri			X						X	
C. butyricum		X			X					
C. cadaveris	X		X							
C. chauvoei	X									
C. difficile										X
C. hastiforme							X			
C. indolis	X							X		
C. malenominatum	X									
C. paraputrificum	X									
C. perenne					X					
C. ramosum						X				
C. septicum	X									
C. sordellii			X							
C. sporogenes							X	XX		
C. tertium							XXX			X

TABLE 3. *Minimal inhibitory concentrations of minocycline*

Organism	Strains tested	Cumulative percentages at increasing concn (μg/ml)								
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25
Bacteroides fragilis	70	29			36	49	66	89	97	100
B. melaninogenicus	22	50			55	65	75	85	100	
Bacteroides sp.	2	100								
Clostridium perfringens	25	64	72					80	100	
Clostridium sp.	26	69		81				88	100	
Fusobacterium sp.	7	71	100							

TABLE 4. *Minimal inhibitory concentrations of metronidazole*

Organism	Strains tested	Cumulative percentages at increasing concn (μg/ml)							
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5
Bacteroides fragilis	70	1	3	4	7	29	43	83	100
B. melaninogenicus	20	15	20	25	30	45	65	95	100
Bacteroides sp.	2	100							
Clostridium perfringens	25					28	56	92	100
Clostridium sp.	26	12	35	38	58	62	69	96	100
Fusobacterium sp.	7	14	29	43	71	86	100		

TABLE 5. *Minimal inhibitory concentrations of penicillin*

Organism	Strains tested	Cumulative percentages at increasing concn (μg/ml)									
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50
Bacteroides fragilis	69	1			3			7	36	94	100
B. melaninogenicus	18	50			55	61	72	83	89	94	100
Bacteroides sp.	2		50	100							
Clostridium perfringens	24	79	83	96					100		
Clostridium sp.	23	35	61		65	78	83	87	96	100	
Fusobacterium sp.	7	43		57	86		100				

TABLE 6. *Minimal inhibitory concentrations of carbenicillin*

Organism	Strains tested	Cumulative percentages at increasing concn (μg/ml)											
		0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	200
Bacteroides fragilis	69					1	4	6	19	56	84	99	100
B. melaninogenicus	17	39	50			55	83	90	100				
Bacteroides sp.	1				100								
Clostridium perfringens	24		33	79	96				100				
Clostridium sp.	23	9			39	52	57	61	70	74	83	91	100
Fusobacterium sp.	7		14		29	100							

gilis (2, 3, 5, 7, 10, 11, 16, 18, 19, 27, 32). This report of the susceptibilities of 70 strains of *B. fragilis* isolated recently from clinical material substantiates the published data and also demonstrates essentially the same pattern of susceptibility as was noted in this laboratory (18) with organisms isolated 3 years ago. Only one strain of *B. fragilis* tested failed to show inhibition at 3.1 μg/ml, a concentration that is readily attainable in serum with this drug. As in the previous study, clindamycin was very active against all strains of *B. melaninogenicus*, *C. perfringens*, and *Fusobacterium* tested.

Although 94% of the *Clostridium* species excluding *C. perfringens* were susceptible to clindamycin at 3.1 μg/ml in 1971, in the present study only 58% of the same group were inhibited by this concentration of drug. However, the

distributions of the various clostridial species were different in the two studies, so all that can be stated is that 42% of the species of *Clostridium* other than *C. perfringens* in this study were resistant to clindamycin in vitro. Most notable among this group were *C. tertium* and *C. sporogenes*, all strains of which had MIC values greater than 3.1 μg/ml. In a study of abdominal trauma by Thadepalli et al. (27), of the two septicemias occurring in patients receiving clindamycin and kanamycin, one involved a mixed *C. perfringens-C. tertium* infection. Tally et al. (25a) reported that only 11 of 24 strains of *C. ramosum* were susceptible to clindamycin. Few studies of the susceptibilities of clinical isolates of *Clostridium* to clindamycin are available (17, 21, 30). There seems to be general agreement that clindamycin is active against *C. perfringens*, but the question of its activity against other clostridial species merits further study.

An increasing resistance of *B. fragilis* to tetracycline has been described by several authors (5, 15, 17–19, 23, 32). A derivative of it, minocycline, was found to be approximately twice as active against *B. fragilis* in this study as was tetracycline in the study by Martin et al. (17). Against the other organisms in this study, the activity of minocycline was comparable to that of tetracycline in the former study. If one accepts the recommendation of the National Committee for Clinical Laboratory Standards Subcommittee on Antimicrobial Susceptibility Testing (1) that an MIC of 6.2 μg/ml be considered indicative of intermediate susceptibility for the tetracyclines, then 80 to 89% of the strains of *Bacteroides* and *Clostridium* tested here would be so classified with respect to minocycline. The present data on the activity of minocycline against *C. perfringens* differ from those of Sapico et al. (21) who found that 100% of the *C. perfringens* strains tested were inhibited at 0.2 μg/ml. This concentration inhibited 72% of the strains tested here with the remaining 28% requiring at least 6.2 μg/ml for inhibition.

Metronidazole, the drug of choice for trichomoniasis and amoebiasis (9, 20), has recently been shown to be effective against anaerobic bacteria in vitro and in limited clinical trials (26). Whelan and Hale (29) and Nastro and Finegold (19) demonstrated that, in addition to being bacteriostatic, metronidazole is also bactericidial in vitro against anaerobic bacteria. The MIC values of metronidazole in this study were higher than those reported but still are within easily achievable serum levels. According to Davies (6), a dosage of 1 g four times a day will provide serum levels of 15 to 75.5 μg/ml. Whelan and Hale (29) state that a single 2-g dose will provide a serum level of 46 μg/ml. All anaerobes tested here were inhibited by metronidazole at 12.5 μg/ml or less. At present, administration of this agent is limited to the oral route. The apparent bactericidal activity of this drug against anaerobic bacteria should encourage further clinical trials.

Our results with penicillin are similar to those of other workers (5, 14, 18, 21, 24, 32), including the earlier study from this laboratory (18), except that, as with clindamycin, clostridia other than *C. perfringens* required significantly higher concentrations for inhibition than they did 3 years ago. In general, the organisms in this group that showed in vitro resistance to clindamycin were susceptible to both penicillin and minocycline. Keusch and O'Connell (15) suggested that, with the parenteral administration of large doses of penicillin G, the criteria for susceptibility proposed by Weinstein et al. (28) might be applicable: inhibition by 78 units or approximately 50 μg/ml or less indicates susceptibility. By this criterion, all organisms we tested would be susceptible, but Bodner et al. (4) reported disappointing clinical results in several patients treated with high doses of penicillin for *Bacteroides* bacteremia. The present availability of effective antimicrobial agents, such as clindamycin or chloramphenicol (2, 4, 5, 10, 16, 17, 27), seems to make unnecessary high-dose penicillin therapy for coverage of *Bacteroides* in sepsis of unknown cause.

Nearly all of the strains tested were inhibited by carbenicillin at 100 μg/ml, a concentration considered to be indicative of susceptibility of *Pseudomonas aeruginosa* (1). This antibiotic is frequently used in the initial therapy for sepsis of unknown cause, because of its activity against *P. aeruginosa* and some species of *Enterobacteriaceae*, and it would be useful to know whether or not its activity against anaerobes in vivo is like that demonstrated in vitro.

Clindamycin was highly effective against the majority of anaerobic isolates tested with the exception of *Clostridium* species other than *C. perfringens*. Because *B. fragilis* is the predominant anaerobe involved in clinically significant anaerobic bacteremia (31), clindamycin and chloramphenicol (not tested in this study) remain the drugs of choice for serious anaerobic infections. Minocycline was more effective than tetracycline against *B. fragilis*, but it failed to inhibit one-third of this group of organisms at expected peak serum levels of 3.1 μg/ml. After further clinical trials and the development of a parenteral form of the drug, metronidazole may

be a promising agent for use in anaerobic infections. High doses of penicillin or carbenicillin for treatment of infections due to *B. fragilis* are of questionable value in view of the availability of more effective agents; however, penicillin remains the drug of choice for treatment of most anaerobic infections other than those due to *B. fragilis*.

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