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Comparative Susceptibility of Anaerobic Bacteria to Ticarcillin, Cefoxitin, Metronidazole, and Related Antimicrobial Agents

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The in vitro antimicrobial activity of two newer agents, ticarcillin and cefoxitin, against 204 recent clinical isolates of anaerobic bacteria was determined by an agar dilution technique, and compared to their related compounds carbenicillin, cephalothin, and penicillin, as well as metronidazole, clindamycin, and chloramphenicol. Ticarcillin was similar to carbenicillin, and cefoxitin was more active than cephalothin. At readily achievable blood concentrations of each antimicrobial agent, ticarcillin was slightly less active than clindamycin against *Bacteroides fragilis*. Cefoxitin was superior to cephalothin and penicillin against *B. fragilis*. Penicillin remained highly active against all obligate anaerobes other than *B. fragilis*. Metronidazole, while highly effective against *B. fragilis* and *Clostridium perfringens*, was only intermediately effective against anaerobic gram-positive cocci and relatively inactive against nonsporulating anaerobic gram-positive bacilli. Further evaluation of the clinical efficacy of ticarcillin, carbenicillin, and cefoxitin against anaerobic infections is indicated.

With the increasing awareness of potential serious side effects of clindamycin (3, 15, 18) and chloramphenicol (16), effective and nontoxic alternative agents are clearly needed for the therapy of severe anaerobic infections.

We evaluated the in vitro activity of two newer antimicrobial agents, ticarcillin and cefoxitin, against 204 recent clinical isolates of obligate anaerobes and compared these results to their related compounds, carbenicillin, cephalothin, and penicillin, as well as to clindamycin, chloramphenicol, and metronidazole. Both of these newer agents have been reported to have broad spectrum activity against obligate anaerobes (5, 10, 14); however, only a limited number of strains has been studied previously.

MATERIALS AND METHODS

The isolates examined in this study, obtained from inpatients of Harbor General Hospital 1971 through 1975, are listed according to genus, species, and subspecies in Table 1. Identification and speciation were determined in prereduced anaerobically sterilized (PRAS) differential media, according to the method of Holdeman and Moore (6). A pH meter was used for recording carbohydrate fermentation reactions, and fatty acid analysis of fermentation products was performed with the AnaBac gas-liquid chromatograph. Clinical isolates were stored in 20% skim milk and frozen at −75°C until ready for susceptibility testing.

Susceptibility testing for all antimicrobial agents was determined on Mueller-Hinton agar (Difco), which had been adjusted to pH 7.2, and enriched with 10% defibrinated sheep blood and 0.01% (vol/vol) vitamin K-hemin. Twofold serial dilutions of ticarcillin (Beecham-Massengil), carbenicillin (Roerig), cefoxitin (Merck, Sharp & Dohme), cephalothin (Lilly), penicillin (USP), clindamycin (Upjohn), chloramphenicol (USP), and metronidazole (Searle) sensitivity powders were added. Test organisms in skim milk were inoculated into PRAS chopped-meat glucose, streaked for purity and growth characteristics, and subcultured in PRAS thioglycolate glucose broth. This subculture was incubated for 48 h and adjusted to a MacFarland no. 1 nephelometer standard (1), previously determined to approximate 10^6 to 10^7 organisms per ml by colony counting in roll tubes with PRAS media. Inocula (0.0025 ml) were delivered with a Steers replicating apparatus (13). Plates were then incubated at 37°C in anaerobic jars after air had been evacuated and replaced with a gas mixture containing 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. Anaerobic, microaerophilic (incubated in a candle jar), and aerobic plates without antibiotics were used for controls, and a reference strain with known minimum inhibitory concentrations (MIC) was included in each test to demonstrate reproducibility. All results were read at 48 h, and the MIC recorded was the least antimicrobial concentration that yielded no visible growth.

RESULTS

The median and range of MIC of all eight antimicrobial agents against various obligate anaerobes tested are summarized in Table 2. Due to paucity of strains representing certain species or subspecies of isolates, data have been grouped and analyzed according to genus designation for most isolates. Relative activities of various antimicrobial agents (expressed as ra-

TABLE 1. *Genera and species of 204 obligate anaerobes tested*

Organism	No. tested	Organism	No. tested
Bacteroides fragilis	85	*Peptococcus*	37
subsp. fragilis	29	*P. asaccharolyticus*	13
subsp. vulgatus	17	*P. prevotii*	12
subsp. thetaiotaomicron	16	*P. magnus*	12
subsp. distasonis	13	*Peptostreptococcus*	29
subsp. ovatus	5	*P. anaerobius*	14
"Other"	5	*P. micros*	13
Bacteroides (not *fragilis*)	15	*P. intermedius*	2
B. melaninogenicus	8	*Clostridium perfringens*	9
B. capillosus	3	*Actinomyces naeslundii*	4
B. oralis	1	*Propionibacterium acnes*	8
B. corrodens	1	*Eubacterium*	11
B. amylophilus	1	*E. lentum*	10
B. pneumosintes	1	*E. aerofaciens*	1
Fusobacterium	3	*Veillonella parvula*	3
F. nucleatum	2		
F. naviforme	1		

TABLE 2. *Ranges of susceptibility of anaerobic bacteria to selected antimicrobial agents*

Genus/species (no. of isolates tested)	Median minimum inhibitory concn and range (μg/ml)			
	Ticarcillin	Carbenicillin	Cefoxitin	Cephalothin
Bacteroides fragilis (85)	34.9 (≤3.1-800)	31.6 (6.2-800)	7.7 (2-≥128)	81.3 (8-≥128)
Bacteroides (miscellaneous) (15)	≤1.8 (≤3.1-6.2)	≤1.8 (≤3.1-6.2)	0.5 (≤0.25-16)	3.0 (0.5-≥128)
Fusobacterium (3)	≤3.1 (≤3.1-6.2)	≤3.1 (≤3.1-6.2)	≤0.25 (≤0.25-2)	0.75 (0.5-1)
Peptococcus (37)	≤1.6 (≤3.1)	≤1.7 (≤3.1-12.5)	0.31 (≤0.25-64)	0.56 (≤0.25-≥128)
Peptostreptococcus (29)	≤1.9 (≤3.1-50)	≤1.9 (≤3.1-50)	0.67 (≤0.25-32)	≤0.25 (≤0.25-16)
Clostridium (9)	≤1.9 (≤3.1-12.5)	≤1.9 (≤3.1-12.5)	0.42 (≤0.25-2)	0.75 (≤0.25-2)
Actinomyces (4)	≤1.5 (≤3.1)	≤1.5 (≤3.1)	2.0 (0.5-32)	4.0 (2-≥128)
Eubacterium (11)	≤2.7 (≤3.1-25)	≤3.1 (≤3.1-25)	2.0 (≤0.25-8)	1.0 (≤0.25-≥128)
Propionibacterium (8)	≤1.5 (≤3.1)	≤1.5 (≤3.1)	1.3 (0.5-4)	0.30 (≤0.25-0.5)
Veillonella (3)	9.3 (6.2-12.5)	12.5 (6.2-25)	0.5 (≤0.25-4)	0.5 (≤0.25-16)
	Penicillin	Clindamycin	Chloramphenicol	Metronidazole
Bacteroides fragilis (85)	14.4 (≤0.16->80)	0.69 (≤0.02-≥10)	8.6 (≤0.1-25)	0.88 (≤0.2-≥100)
Bacteroides (miscellaneous) (15)	≤0.13 (≤0.16->80)	≤0.02 (≤0.02-5.0)	4.3 (≤0.1-50)	0.64 (≤0.2-≥100)
Fusobacterium (3)	≤0.10 (≤0.16)	0.06 (≤0.02-0.08)	1.2(≤0.1-1.6)	≤0.13 (≤0.20)
Peptococcus (37)	≤0.12 (≤0.16-20)	0.38 (≤0.02->10)	3.1(≤0.1-25)	0.71 (≤0.2-≥100)
Peptostreptococcus (29)	≤0.10 (≤0.16-10)	0.05 (≤0.02-0.62)	1.3(≤0.1-12.5)	2.3 (≤0.2-≥100)
Clostridium (9)	≤0.10 (≤0.16-0.31)	1.25 (≤0.02-5.0)	4.0(1.6-12.5)	0.67 (≤0.2-1.6)
Actinomyces (4)	≤0.16 (≤0.16-0.62)	0.31 (≤0.02-1.25)	2.4(0.8-6.25)	25.0 (0.8-≥100)
Eubacterium (11)	≤0.21 (≤0.16-1.25)	0.08 (≤0.02-1.25)	4.0(≤0.1-12.5)	1.2 (≤0.2-≥100)
Propionibacterium (8)	≤0.08 (≤0.16)	0.08 (≤0.02-1.25)	2.7(0.8-6.25)	>100 (>100)
Veillonella (3)	≤0.08 (≤0.16)	0.12 (≤0.02-0.16)	9.4(0.8-6.25)	9.4 (≤0.2-12.5)

tios of antimicrobial concentrations readily achievable in plasma to concentrations required to inhibit growth of 90% of isolates) are also summarized in Table 3.

Against *B. fragilis*, ticarcillin was equally active as carbenicillin, with a similar median MIC and comparable relative activity. Cefoxitin was far superior to cephalothin. At readily achieved blood concentrations, the relative activity of ticarcillin against *B. fragilis* was slightly less active than clindamycin and metronidazole, but higher than the remaining agents.

With the exception of cephalothin and metronidazole, relative activities of all antimicrobial agents tested were more than adequate against obligate anaerobes other than *Bacteroides*.

Metronidazole demonstrated variable activity against obligate anaerobes other than *B. fragilis*, and was least active against *Propionibacterium*, *Actinomyces*, *Peptostreptococcus*, and *Peptococcus*.

DISCUSSION

Recent awareness of the potential serious complications of clindamycin (3, 8, 15) as well as chloramphenicol (16) has prompted an intensive search for alternative antimicrobial agents with excellent activity against obligate anaerobes, especially *B. fragilis*. Additionally, an increasing number of obligate anaerobes has been noted to be resistant to chloramphenicol (7) as well as clindamycin (12). For these reasons, the in vitro activity of several potentially useful, newer antimicrobial agents was tested and compared.

Carbenicillin has been reported as an effective and relatively safe agent in the therapy of certain anerobic infections (4). In the present study, however, it was slightly less active than clindamycin and metronidazole against *B. fragilis* at readily achievable blood concentrations.

Ticarcillin, a newer semisynthetic penicillin, which has been demonstrated to be more active in vitro against certain aerobic gram-negative bacilli than carbenicillin (2, 9), also appears to be comparable to carbenicillin for all species of obligate anaerobes tested. With respect to *B. fragilis*, ticarcillin was slightly less active than clindamycin and metronidazole, but was more active than chloramphenicol, cefoxitin, cephalothin, and penicillin.

Cefoxitin, while inferior to ticarcillin against *B. fragilis* in vitro, was comparable to ticarcillin, carbenicillin, clindamycin and chloramphenicol against the remaining obligate anaer-

TABLE 3. *Relative activities of various antimicrobial agents against diverse genera and species of anaerobic bacteria*[a]

Genus/species (No. of isolates tested)		RACP/MIC$_{90}$ (μg/ml)[b]			
		Ticarcillin	Carbenicillin	Cefoxitin	Cephalothin
Bacteroides fragilis	(85)	100/97.7 (1.0)	100/95.8 (1.0)	16/25.8 (0.6)	16/>128 (<0.1)
Bacteroides (misc.)	(15)	100/≤3.0 (≥33.3)	100/≤3.0 (≥33.3)	16/10.0 (1.6)	16/>128 (<0.1)
Fusobacterium	(3)	100/5.7 (17.5)	100/5.7 (17.5)	16/1.7 (9.4)	16/0.92 (17.3)
Peptococcus	(37)	100/≤2.8 (≥35.7)	100/≤3.0 (≤33.3)	16/3.3 (4.8)	16/10.4 (1.5)
Peptostreptococcus	(29)	100/6.2 (16.1)	100/9.6 (10.4)	16/6.7 (2.3)	16/2.1 (7.6)
Clostridium	(9)	100/4.04 (24.8)	100/4.04 (24.8)	16/1.1 (14.5)	16/1.7 (9.4)
Actinomyces	(4)	100/≤2.8 (≥35.7)	100/≤2.8 (≥35.7)	16/25.6 (0.6)	16/>128 (<0.1)
Eubacterium	(11)	100/9.0 (11.1)	100/12.1 (8.2)	16/5.8 (2.7)	16/30.4 (0.5)
Propionibacterium	(8)	100/≤2.8 (≥35.7)	100/≤2.8 (≥35.7)	16/3.2 (5.0)	16/0.46 (34.7)
Veillonella	(3)	100/11.5 (8.7)	100/21.2 (4.7)	16/3.4 (4.7)	16/13.6 (1.1)
		Penicillin	Clindamycin	Chloramphenicol	Metronidazole
Bacteroides fragilis	(85)	16/55 (0.3)	5/2.2 (2.3)	12.5/16.8 (0.7)	12.5/3.6 (3.5)
Bacteroides (misc.)	(15)	16/30 (0.5)	5/0.47 (10.6)	12.5/10.9 (1.1)	12.5/>100 (<0.1)
Fusobacterium	(3)	16/≤0.14 (≥114.3)	5/0.07 (71.4)	12.5/1.48 (8.4)	12.5/≤0.18 (≥69.4)
Peptococcus	(37)	16/0.57 (28.0)	5/3.3 (1.5)	12.5/9.7 (1.3)	12.5/>100 (<0.1)
Peptostreptococcus	(29)	16/≤0.14 (≥114.3)	5/0.24 (20.8)	12.5/4.4 (2.8)	12.5/>100 (<0.1)
Clostridium	(9)	16/0.24 (66.6)	5/2.8 (1.8)	12.5/6.9 (1.8)	12.5/1.36 (9.2)
Actinomyces	(4)	16/0.50 (32.0)	5/1.1 (4.5)	12.5/5.1 (2.4)	12.5/>100 (<0.1)
Eubacterium	(11)	16/0.60 (26.6)	5/0.91 (5.5)	12.5/9.1 (1.4)	12.5/95 (0.1)
Propionibacterium	(8)	16/≤0.15 (≥106.6)	5/0.75 (6.6)	12.5/5.5 (2.3)	12.5/>100 (<0.1)
Veillonella	(3)	16/≤0.14 (≥114.3)	5/0.15 (33.3)	12.5/11.6 (1.1)	12.5/11.6 (1.1)

[a] Expressed as ratios of concentrations readily obtained in plasma (RACP) to concentrations required to inhibit growth of 90% of isolates (MIC$_{90}$).

[b] Figure in parenthesis, calculated index.

obes tested. Its strikingly improved activity compared with cephalothin and penicillin is undoubtedly related to its resistance against beta-lactamase of *B. fragilis* (10, 11).

These in vitro data indicate that ticarcillin, carbenicillin, and cefoxitin may be useful alternative agents for clindamycin and chloramphenicol in the treatment of serious anaerobic infections, and further clinical studies with these agents are clearly warranted.

LITERATURE CITED

1. **Bailey, W. R., and E. G. Scott (ed.).** 1970. Diagnostic Microbiology. C. V. Mosby, St. Louis.
2. **Bodey, G. P., and B. Deerhake.** 1971. In vitro studies of α-carboxyl-3-thienylmethyl penicillin, a new semisynthetic penicillin. Antimicrob. Agents Chemother. 21:61–65.
3. **Cohen, L. E., C. J. McNeill, and R. F. Wells.** 1973. Clindamycin-associated colitis. J. Am. Med. Assoc. 223:1379–1380.
4. **Fiedelman, W., and C. C. Webb.** 1975. Clinical evaluation of carbenicillin in the treatment of infection due to anaerobic bacteria. Curr. Ther. Res. 18:441–451.
5. **Finegold, S. M., J. G. Bartlett, A. W. Chow, D. J. Flora, S. L. Gorbach, E. J. Harder, and F. P. Tally.** 1975. Management of anaerobic infections. Ann. Intern. Med. 83:375–389.
6. **Holdeman, L. V., and W. E. C. Moore (ed.)** 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute Anaerobe Laboratory, Blacksburg.
7. **Kagnoff, M. F., and D. Armstrong.** 1971. Antibiotics for Bacteroides sepsis (letter to the editor). Ann. Intern. Med. 75:316–317.
8. **Keusch, G. T., and D. H. Present.** 1976. Summary of a workshop of clindamycin colitis. J. Infect. Dis. 133:578–588.
9. **Neu, H. C., and E. B. Winshell.** 1971. In vitro studies of a semisynthetic penicillin 6-[D(-)-α-carboxy-3-thienylacetamido] penicillin acid (BRL 2288), active against *Pseudomonas*, p. 385–389. Antimicrob. Agents Chemother. 1970.
10. **Norrby, R., J. E. Brorsson, and S. Seeberg.** 1976. Comparative study of the in vitro antibacterial activity of cefoxitin, cefuroxime, and cephaloridine. Antimicrobe. Agents Chemother. 9:506–510.
11. **Onishi, H. R., D. R. Daoust, S. B. Zimmerman, D. Henoun, and E. O. Staphley.** 1974. Cefoxitin, a semisynthetic antibiotic resistant to beta-lactamase inactivation. Antimicrob. Agents Chemother. 5:38–48.
12. **Salaki, J. S., R. Black, F. P. Tally, and J. W. Kislak.** 1976. *Bacteroides fragilis* resistant to the administration of clindamycin. Am. J. Med. 60:426–428.
13. **Steers, E., E. L. Foltz, B. S. Graves, and J. Riden.** 1959. Inocula-replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9:307–311.
14. **Tally, F. P., N. V. Jacobus, J. G. Bartlett, and S. L. Gorbach.** 1975. Susceptibility of anaerobes to cefoxitin and other cephalosporins. Antimicrob. Agents Chemother. 7:128–132.
15. **Tedesco, F. J., R. W. Barton, and D. H. Alders.** 1974. Clindamycin-associated colitis, a prospective study. Ann. Intern. Med. 81:429–433.
16. **Wallerstein, R. O., P. K. Conoit, and C. K. Kasper.** 1969. Statewide study of chloramphenicol therapy and fatal aplastic anemia. J. Am. Med. Assoc. 208:2045–2050.