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Antimicrobial Susceptibility of *Capnocytophaga*

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Capnocytophaga (*Bacteroides ochraceus*, Center for Disease Control biogroup DF-1) is associated with sepsis in granulocytopenic patients and is isolated in large numbers from the affected periodontal pockets in patients with juvenile periodontosis. The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of 17 antimicrobial agents for 13 strains of *Capnocytophaga* organisms were determined. In addition, the ratio of the MBC to the MIC for each antimicrobial agent was determined for each strain. At concentrations of 1 μg/ml or less, penicillin, ampicillin, carbenicillin, erythromycin, and clindamycin killed 90% of the strains. At concentrations of 3.12 μg/ml or less, tetracycline, metronidazole, cefoxitin, and chloramphenicol killed 90% of the strains. None of the aminoglycosides tested demonstrated antibacterial activity at 50 μg/ml. Penicillin, ampicillin, carbenicillin, and cefoxitin exhibited MBC/MIC ratios of 4 or less with all strains. Erythromycin, tetracycline, and metronidazole exhibited MBC/MIC ratios of 4 or less for 12 of 13 strains. The MICs of cephalothin and cefazolin for 90% of the strains were 25 and 50 μg/ml, respectively. The MBC/MIC ratios for these drugs were 4 or less for 12 of 13 and 7 of 13 strains, respectively. The MIC of cefamandole for 90% of the strains was 3.12 μg/ml; however, only nine strains had an MBC/MIC ratio of 4 or less.

The generic designation *Capnocytophaga* has recently been given to a group of gram-negative, gliding bacilli which are part of the normal oral flora and have been isolated in large numbers from the periodontal lesions of patients with localized juvenile periodontitis (4). These organisms are facultatively anaerobic, capnophilic, fusiform, and dysgonically fermenting rods. Newman et al. (5) and Williams et al. (7) demonstrated in 1979 the synonymy of *Capnocytophaga, Bacteroides ochraceus*, and Center for Disease Control biogroup DF-1.

We recently documented *Capnocytophaga* as the cause of sepsis in patients with malignancy complicated by profound granulocytopenia and oral mucositis (3). Since *Capnocytophaga* organisms appear to be opportunistic pathogens in immunosuppressed patients, and since few data are available regarding their antimicrobial susceptibility, we determined the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of 10 clinical isolates and three reference strains of *Capnocytophaga* to 17 antimicrobial agents.

MATERIALS AND METHODS

Bacteriological studies. Organisms were identified both by conventional methods (6) and by the buffered single substrate technique described by Blachman and Pickett (1). Six isolates were recovered from the blood of as many patients with granulocytopenia and malignancy. Four isolates were obtained from the periodontal pockets of patients seen in the Periodontal Clinic, University of California-Los Angeles School of Dentistry. Three strains were stock cultures of *Capnocytophaga sputigena, C. ochracea*, and *C. gingivalis*, provided by S. S. Socransky (Forsyth Dental Institute, Boston, Mass.).

Antimicrobial susceptibility studies. Antibiotics were obtained from the following sources: ampicillin, penicillin G, cefazolin, and tetracycline hydrochloride from Smith Kline & French, Philadelphia, Pa.; sodium oxacillin and amikacin sulfate from Bristol Laboratories, East Syracuse, N.Y.; cephalothin sodium, cefamandole nafate, vancomycin hydrochloride, and tobramycin sulfate from Eli Lilly & Co., Indianapolis, Ind.; clindamycin phosphate from The Upjohn Co., Kalamazoo, Mich.; chloramphenicol from Parke-Davis, Morris Plains, N.J.; cefoxitin sodium from Merck & Co., Inc., Rahway, N.J.: carbenicillin disodium from Roerig, New York, N.Y.; metronidazole from Searle and Co., San Juan, P.R.; erythromycin from Abbott Laboratories, North Chicago, Ill.; and gentamicin sulfate from Schering Corp., Kenilworth, N.J. Solutions of these antibiotics in distilled water were filter sterilized and frozen in 2-ml samples at −4°C for up to 3 weeks. Trypticase soy agar and 5% sheep blood plates were kindly donated by BBL Microbiology Systems (Cockeysville, Md.) and Scientific Products, Inc. (Irvine, Calif.), respectively.

MIC and MBC were determined by a modification of a standard technique (2). Serial twofold dilutions of each antimicrobial agent were made in Trypticase soy

broth (BBL) supplemented with 1% dextrose, a medium which consistently supported luxuriant growth for all strains of organisms tested. Concentrations tested were: carbenicillin, 0.5 to 100 μg/ml; metronidazole, 0.25 to 64 μg/ml; and all other agents, 0.2 to 50 μg/ml. The inoculum consisted of a 100-fold dilution of a 48-h anaerobic broth culture of the test organisms (ca. 10^7 colony-forming units per ml). The MIC was defined as the lowest antibiotic concentration at which no turbidity was visible after 48 h of anaerobic incubation. The MBC was determined by inoculating 0.01 mg of broth from each clear tube onto blood agar plates (BBL). The MBC was designated as the lowest antibiotic concentration at which these plates were free of bacterial growth after incubation for 48 h in an anaerobic chamber. Appropriate antibiotic sterility and bacterial growth controls were included in all studies.

RESULTS

Penicillin, ampicillin, carbenicillin, erythromycin, and clindamycin were the most active agents, being bactericidal for 90% of the isolates at concentrations of 1 μg/ml or less (Table 1). Tetracycline, metronidazole, cefoxitin, and chloramphenicol ranked next in bactericidal activity, followed by oxacillin, cephalothin, cefazolin, cefamandole, and vancomycin. None of the aminoglycosides tested exhibited activity at a concentration of 50 μg/ml.

The ratios of the MBCs to the MICs for the various drugs for each of the 13 isolates are shown in Table 2. Isolates with MICs or MBCs less than the lowest antibiotic concentration in the test medium were designated as having ratios of 1. Ratios were listed as not calculable when MICs or MBCs exceeded the highest concentration of drug in the test medium. Penicillin, ampicillin, carbenicillin, and cefoxitin exhibited MBC/MIC ratios of ≤4 for all strains. Erythromycin, tetracycline, and metronidazole demonstrated bactericidal activity against 12 of 13 strains. Although oxacillin and cephalothin exhibited relatively high MIC and MBC values, each exhibited an MBC/MIC ratio of ≤4 against 12 of the 13 strains. The MBC/MIC ratios were not obtainable for the aminoglycosides. All other antimicrobial agents did not exhibit significant or consistent bactericidal activity.

TABLE 2. *MBC/MIC ratios of antimicrobial agents against 13 strains of Capnocytophaga*

Drug	No. of strains with MBC/MIC ratio:						
	1	2	4	8	16	>16	NC[a]
Penicillin	11	2					
Ampicillin	11	1	1				
Oxacillin	7	3	1		1	1	
Carbenicillin	11	2					
Cephalothin	7	3	2				1
Cefazolin	3	2	2	1			5
Cefamandole	1	3	5	2	1	1	
Cefoxitin	7	5	1				
Erythromycin	7	2	3				1
Tetracycline	10	1	1	1			
Chloramphenicol	3	3	4	1	1		1
Clindamycin	13						
Metronidazole	5	4	3		1		
Vancomycin		2	3	2	2	3	1
Amikacin							13
Gentamicin							13
Tobramycin							13

[a] NC, Not calculable due to high level of resistance.

TABLE 1. *Antimicrobial susceptibility of 13 strains of Capnocytophaga*

Drug	MIC (μg/ml)			MBC (μg/ml)		
	Range	For % of strains:		Range	For % of strains:	
		50	90		50	90
Penicillin	<0.20–0.39	<0.20	<0.20	<0.20–0.78	<0.20	0.20
Ampicillin	<0.20–0.39	<0.20	<0.20	<0.20–0.78	<0.20	0.39
Oxacillin	0.78–25.00	6.25	25.00	6.25–25.00	25.00	25.00
Carbenicillin	<0.50–2.00	<0.50	<0.50	<0.50–2.00	<0.50	1.00
Cephalothin	1.56–>50.00	12.50	25.00	3.12–>50.00	12.50	50.00
Cefazolin	0.78–>50.00	6.25	50.00	3.12–>50.00	12.50	>50.00
Cefamandole	<0.20–25.00	0.78	3.12	0.78–>50.00	3.12	50.00
Cefoxitin	<0.20–6.25	0.39	1.56	<0.20–6.25	0.78	3.12
Erythromycin	<0.20–50.00	<0.20	0.78	<0.20–>50.00	<0.20	0.78
Tetracycline	<0.20–1.56	<0.20	0.78	<0.20–1.56	0.20	1.56
Chloramphenicol	<0.20–6.25	0.39	6.25	0.78–12.50	6.25	6.25
Clindamycin	<0.20–0.39	<0.20	<0.20	<0.20–0.39	0.20	<0.20
Metronidazole	<0.25–8.00	<0.25	2.00	<0.25–8.00	0.50	2.00
Vancomycin	<0.20–50.00	3.12	25.00	12.50–>50.00	50.00	50.00
Amikacin	12.50–>50.00	>50.00	>50.00	all–>50.00	>50.00	>50.00
Gentamicin	25.00–>50.00	>50.00	>50.00	all–>50.00	>50.00	>50.00
Tobramycin	all–>50.00	>50.00	>50.00	all–>50.00	>50.00	>50.00

DISCUSSION

The recognition of *Capnocytophaga* as a cause of sepsis in granulocytopenic patients with malignancies underlines the need for data regarding their antimicrobial susceptibility. Since bactericidal drugs are considered beneficial in this patient population, particular attention has been given to the bactericidal activity as measured by the MBC/MIC ratios of the antimicrobial agents for these organisms. Our data indicate that antimicrobial agents known to be active against anaerobic organisms have the greatest activity against *Capnocytophaga*. Of those drugs, penicillin, ampicillin, carbenicillin, and clindamycin also possess bactericidal activity. Of those agents commonly instituted empirically for suspected sepsis in granulocytopenic patients, carbenicillin has a predictable antimicrobial activity with a low MIC, MBC, and MBC/MIC ratio. With the exception of cefoxitin, the cephalosporins did not demonstrate consistent antimicrobial activity.

Our findings suggest that clindamycin, penicillin, ampicillin, or carbenicillin would be the antibiotic of choice in the therapy of documented *Capnocytophaga* sepsis. We recommend that one of these agents should be included in the empiric antimicrobial regimen used to treat suspected sepsis in granulocytopenic patients with oral mucositis or periodontal disease.

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