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Minimal Inhibitory Concentrations of Various Antimicrobial Agents for Human Oral Anaerobic Bacteria

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The minimal inhibitory concentrations of a series of antimicrobial agents for human oral organisms were determined under anaerobic growth conditions by an agar dilution assay. With the exception of black-pigmented *Bacteroides* spp., minimal inhibitory concentrations for oral isolates were similar to those for non-oral isolates of organisms of the same or closely related species.

Bacteria indigenous to the human oral cavity can cause various clinical infections. *Streptococcus mutans* and *Lactobacillus* and *Actinomyces* spp. are associated with supragingival plaque leading to dental caries (11) and gingivitis (22). *Fusobacterium nucleatum, Eikenella corrodens*, and corroding *Bacteroides* spp., *Capnocytophaga* spp., and anaerobic vibrios (22), as well as *Bacteroides gingivalis, Bacteroides melaninogenicus* subsp. *intermedius*, and *Actinobacillus actinomycetemcomitans* (26), are associated with periodontitis. Many orofacial abscesses are mixed anaerobic infections. *B. melaninogenicus, Bacteroides oralis, Bacteroides corrodens*, streptococci (7), and *Actinomyces israelii* (31) have been isolated from periapical abscesses. *A. israelii* and *A. actinomycetemcomitans* are found in actinomycosis (19, 24). *B. melaninogenicus* (3, 7), *B. oralis* (7), *B. corrodens* (7), *Campylobacter* spp., (7), *F. nucleatum* (3, 7), *Veillonella parvula* (7), and streptococci (3, 7) can give rise to infections of the perimandibular fascial spaces. Various *Bacteroides* and *Campylobacter* spp., *V. parvula*, and *Actinomyces* spp. are frequent isolates of mandibular osteomyelitis (7). Organisms also can seed from the oral cavity to other parts of the body and cause clinical infections. Systemic antibiotic therapy is useful in the treatment of infections caused by oral bacteria (2, 4, 10, 12, 15, 24). Few data exist, however, on the antibiotic susceptibilities of oral facultative and anaerobic organisms. Also, it is unclear as to whether oral isolates differ from non-oral isolates in their antimicrobial susceptibilities. In this study we tested the susceptibilities of isolates of the major bacterial species from the human oral cavity to several antimicrobial agents.

Bacteria studied were freshly isolated as previously described (28) from periodontitis patients. Isolated strains were characterized by established procedures (8, 16, 34). Additional strains of human oral origin were obtained from the culture collection maintained at the Periodontal Disease Clinical Research Center, State University of New York at Buffalo. These included *B. gingivalis* 381; *B. melaninogenicus* subsp. *intermedius* 20-3; *Haemophilus aphrophilus* ATCC 13252, ATCC 19415, NTCC 5906, NTCC 5907, and NTCC 5908; *Lactobacillus casei* ATCC 11578, ATCC 11582, and ATCC 4646; *Streptococcus mutans* AHT, BHT, GS5-2, OMZ 175, OMZ 176, LM7, and MT557; and *Streptococcus salivarius* ATCC 9758 and ATCC 13419.

Nomenclature of the *B. melaninogenicus* subspecies is in the process of change on the basis of new knowledge of DNA homology (L. V. Holdeman, personal communication). We have retained the names *B. melaninogenicus* subsp. *intermedius* and *B. melaninogenicus* subsp. *melaninogenicus*, as they are the accepted names as of the time of this writing. The corroding *Bacteroides* spp. are the corroding gram-negative anaerobic rods.

The antibiotics tested were obtained from the following sources: actinobolin sulfate, Calbiochem, Los Angeles, Calif.; spiramycin base, Poulenc Ltd., Montreal, Canada; vancomycin hydrochloride and minocycline hydrochloride, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y.; chloramphenicol sodium succinate, Parke-Davis, Ann Arbor, Mich.; clindamycin hydrochloride, The Upjohn Co., Kalamazoo, Mich.; disodium carbenicillin, Pfizer Inc., New York, N.Y.; erythromycin, penicillin G, tetracycline hydrochloride, and tyrothricin, Sigma Chemical Co., St. Louis, Mo. Erythromycin and tyrothricin were dissolved in ethyl alcohol and spiramycin was dissolved in dimethyl sulfoxide at final solvent concentrations of no more than 0.75%. The solvents did

TABLE 1. Suceptibilities of oral anaerobic bacteria to various antimicrobial agents

Organism (no. of strains)	MIC (μg/ml) of following antimicrobial agent for indicated % of strains:											
	Penicillin G		Carbenicillin		Chloramphenicol		Clindamycin		Erythromycin		Metronidazole	
	50	90	50	90	50	90	50	90	50	90	50	90
Gram-negative												
Actinobacillus actinomyctemcomitans (11)	0.29	2.4	42	>84	0.38	0.75	92	92	9.6	19	2.1	4.3
Bacteroides melaninogenicus												
subsp. *intermedius* (9)	0.15	4.8	0.16	0.66	3.0	96	≤0.011	0.36	1.2	2.4	0.13	1.1
subsp. *melaninogenicus* (7)	0.036	0.072	0.16	0.66	1.5	3.0	0.023	0.023	0.15	1.2	0.53	8.6
Corroding *Bacteroides* spp. (5)	0.15	1.2	0.080	0.16	3.0	3.0	0.36	0.72	0.30	2.4	0.53	0.53
Bacteroides gingivalis (11)	0.072	0.29	0.16	0.32	3.0	24	<0.011	≤0.011	0.30	2.4	0.53	2.1
Bacteroides oralis (7)	0.29	0.29	1.3	>84	>96	>96	0.18	>92	4.8	4.8	ND[a]	ND
Campylobacter spp. (3)	1.2	19	5.6	11	48	48	0.36	11	38	38	ND	ND
Capnocytophaga spp. (8)	0.15	0.59	1.3	>84	6.0	24	≤0.011	0.046	1.2	2.4	0.53	4.3
Eikenella corrodens (5)	1.2	2.4	0.66	1.3	48	48	>92	>92	19	19	ND	ND
Fusobacterium nucleatum (14)	0.036	4.8	0.080	0.66	0.38	24	0.046	5.6	38	77	ND	ND
Haemophilus aphrophilus (6)	0.072	0.59	1.3	2.6	0.75	0.75	46	92	2.4	9.6	34	68
Veillonella parvula (6)	0.072	0.072	0.66	0.66	0.75	1.5	0.090	0.18	77	154	68	68
Anaerobic vibrios (10)	0.15	4.8	0.080	1.3	3.0	48	0.72	2.9	1.2	4.8	0.27	0.27
Gram-positive												
Actinomyces israelii (6)	0.009	0.59	0.080	5.6	0.75	1.5	0.090	0.36	≤0.037	0.074	34	34
Actinomyces naeslundii (11)	0.036	0.072	0.66	1.3	0.75	6.0	0.18	92	≤0.037	0.074	68	68
Actinomyces viscosus (9)	0.009	0.072	0.66	1.3	0.75	6.0	1.4	5.6	≤0.037	0.074	34	34
Lactobacillus casei (3)	1.2	1.2	5.2	5.2	>96	>96	0.36	0.36	0.30	1.2	ND	ND
Streptococcus mutans (7)	0.072	4.8	1.3	1.3	48	>96	0.36	2.9	1.2	38	ND	ND
Streptococcus salivarius (2)	0.072	0.29	0.66	1.3	>96	>96	0.025	0.36	0.30	1.2	ND	ND

[a] ND, Not done.

TABLE 2. Susceptibilities of oral anaerobic bacteria to various antimicrobial agents

Organism (no. of strains)	MIC (μg/ml) of following antimicrobial agent for indicated % of strains:											
	Spiramycin		Tyrothricin		Vancomycin		Actinobolin		Tetracycline		Minocycline	
	50	90	50	90	50	90	50	90	50	90	50	90
Gram-negative												
Actinobacillus actinomycetemcomitans (11)	>175	>175	>300	>300	>660	>660	15	30	1.5	1.5	1.7	3.4
Bacteroides melaninogenicus subsp. *intermedius* (6)	2.8	5.6	0.60	2.3	21	42	15	>120	0.19	6.0	0.053	0.85
subsp. *melaninogenicus* (5)	2.8	5.6	0.60	19	5.2	10	15	30	0.19	0.75	0.053	0.21
Corroding *Bacteroides* spp. (5)	0.70	2.8	38	>300	660	>660	15	30	0.19	0.75	0.21	0.42
Bacteroides gingivalis (9)	2.8	5.6	2.3	9.4	5.2	160	15	60	0.19	1.5	0.026	0.42
Bacteroides oralis (4)	22	>175	19	75	42	160	>120	>120	1.5	24	0.85	3.4
Campylobacter spp. (4)	175	>175	75	300	>660	>660	60	>120	3.0	96	0.42	0.85
Capnocytophaga spp. (9)	2.8	5.6	9.4	19	10	42	60	>120	1.5	12	0.21	0.85
Eikenella corrodens (2)	88	>175	9.4	9.4	160	330	60	120	1.5	3.0	0.85	1.7
Fusobacterium nucleatum (13)	22	175	19	19	330	660	120	>120	0.75	1.5	0.21	0.42
Haemophilus aphrophilus (5)	>175	>175	>300	>300	160	660	15	15	0.75	1.5	0.85	1.7
Veillonella parvula (6)	175	>175	38	300	160	660	30	60	1.5	48	0.85	218
Anaerobic vibrios (10)	28	22	75	>300	330	660	30	60	0.75	6.0	0.21	28
Gram-positive												
Actinomyces israelii (6)	0.17	0.70	2.3	19	0.63	2.6	30	60	0.75	0.75	0.053	0.85
Actinomyces naeslundii (8)	0.35	0.70	19	38	1.3	1.3	30	60	0.75	1.5	0.42	0.85
Actinomyces viscosus (7)	0.70	5.6	2.3	4.6	≤0.63	2.6	30	60	0.75	0.75	0.21	0.85
Lactobacillus casei (3)	44	44	2.3	2.3	>660	>660	>120	>120	1.5	6.0	0.85	0.85
Streptococcus mitior (2)	0.17	0.17	2.3	19	330	330	>120	>120	ND[a]	ND	ND	ND
Streptococcus mutans (7)	11	11	4.6	4.6	5.2	5.2	>120	>120	1.5	1.5	0.85	0.85
Streptococcus salivarius (2)	1.4	11	0.60	4.6	1.3	1.3	>120	>120	0.75	1.5	0.11	0.85

[a] ND, Not done.

not cause bacterial inhibition. All other agents were dissolved in distilled water.

Minimal inhibitory concentrations (MICs) were determined by a standard procedure for agar dilution (18). A series of each agent, diluted twofold, was prepared and incorporated into Wilkins-Chalgren agar (37). The plates were reduced overnight in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) in an atmosphere of 5% CO_2–10% H_2–85% N_2. In such a diverse group of organisms, a particular McFarland standard will not indicate a constant cell mass; therefore, 72-h cultures in Wilkins-Chalgren broth (Wilkins-Chalgren medium minus agar) were adjusted to 10^7 cells per ml. Duplicate plates were inoculated with a Steers replicator (30). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were routinely included as internal controls for reproducibility of the experiments. Incubation took place for 4 days at 37°C in the anaerobic chamber described above. Many of the test organisms grew slowly and, consequently, required 4 days of incubation to obtain good growth on control plates. MICs of the faster-growing organisms were checked after 48 h and did not change between 48 h and 4 days.

Some strains of *B. gingivalis*, *B. melaninogenicus* subsp. *melaninogenicus*, and *B. melaninogenicus* subsp. *intermedius* grew poorly on the agar medium and several other broth and agar media. The susceptibility testing of these strains was performed by an accepted broth macrodilution technique (18), except the tests were done in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) with added hemin (5 μg/ml) and vitamin K (0.5 μg/ml) and incubation was carried out for 4 days, as for the agar dilution tests. Some *Bacteroides* strains and the *E. coli* and *S. aureus* control strains were tested by both agar dilution and broth dilution. The two methods gave similar MICs; therefore, the broth dilution MICs for strains for which MICs could be obtained only by this method were combined with the agar dilution MICs for the other strains.

The lowest concentrations required to inhibit 50 and 90% of the strains are shown in Tables 1 and 2.

The results are generally similar to those previously published for non-oral clinical isolates of these species when tested anaerobically by agar dilution (1, 5, 6, 13–15, 17, 20, 25, 29, 32, 33). Each of these studies contained one or more of the drug organism combinations tested in the present study.

The MICs reported here for oral *B. melaninogenicus*, however, were lower than those reported for non-oral *B. melaninogenicus* strains when tetracycline (1, 5, 6, 14, 20, 21), minocycline (6, 29, 32), penicillin (1, 5, 14, 20, 21, 29), and carbenicillin (1, 14, 29, 32) were considered. Available MICs for oral *B. melaninogenicus*, i.e., those of tetracycline (23, 35) and penicillin (23, 36), were also lower than those reported for non-oral strains. For clindamycin, erythromycin, and metronidazole, the present MICs agree with those previously published for oral (23, 36) and non-oral (1, 14, 20, 21, 29, 32) strains.

The MICs of spiramycin or clindamycin obtained in this study for *A. actinomycetemcomitans* are somewhat higher than those reported for non-oral strains (15) but agree with earlier results on periodontal *A. actinomycetemcomitans* (27). MICs of metronidazole for *Capnocytophaga* spp. agree with those of some previous reports (9, 23) but are lower than those reported by Sutter et al. (33).

Erythromycin MICs for oral *S. mutans* in the present study were higher than those found for *S. mutans* from endocarditis patients (2) but were also higher than those reported for oral streptococci (36). MICs for oral *S. mutans* were not different from those of tetracycline (2) or penicillin (2, 4) for *S. mutans* isolates from endocarditis patients.

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