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Antimicrobial Susceptibilities of Bacteria Associated with Periodontal Disease

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A total of 193 bacterial strains were tested for their susceptibilities to 14 antimicrobial agents. Penicillin G was active at 2 U/ml against 98% of the oral isolates. Other antibiotics with good activity were cefoperazone, moxalactam, Sch 29,482, and clindamycin. Metronidazole was active against more than 90% of the anaerobic bacteria and *Capnocytophaga* but was inactive against most other microaerophilic and facultative strains.

The recognition that destructive and recurrent periodontal diseases are associated with specific bacteria or combinations of bacteria has led to an increased interest in the use of antimicrobial agents in the therapy of these diseases. Recent studies of the in vitro susceptibility of bacteria isolated from oral samples are relatively few and usually present data on small numbers of strains or few antimicrobial agents (1, 6–8, 10, 12–15). The purpose of this study was to determine the in vitro susceptibilities of bacteria associated with periodontal disease to agents which may be useful therapeutically or as selective agents in culture media.

(This paper was presented in part previously [V. L. Sutter, M. J. Jones, and A. T. Ghoneim, Annu. Meet. Am. Soc. Microbiol. 1982, A18, p. 4].)

A total of 193 strains were tested. Most were isolated from subgingival plaque samples from both healthy and diseased sites of outpatients with periodontal disease. The patients had no history of antimicrobial therapy within 6 months before sampling. Bacteria associated with healthy as well as diseased sites were included because it is important to know the susceptibility of the more normal flora as well as the potentially pathogenic flora. The use of antimicrobial agents which would eliminate bacteria such as the streptococci and lactobacilli while allowing the retention of potential pathogens might potentiate the infection rather than eliminate it. Conversely, an agent which would act against potential pathogens while being relatively inactive against the more normal bacteria would be desirable. Three *Bacteroides asaccharolyticus* strains and one *Bacteroides corporis* strain were from clinical sources. Eighteen type or reference strains were also included.

The antimicrobial agents were kindly supplied by the manufacturers.

For the anaerobic bacteria, *Capnocytophaga* spp., *Actinomyces* spp., *Arachnia* spp., *Propionibacterium* spp., and *Lactobacillus* spp., the antimicrobial agents were incorporated into brucella agar supplemented with vitamin K_1 (10 μg/ml) and 5% laked sheep blood. The tests were performed as previously described (9). *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were included as controls with each test run.

For *Eikenella* sp., *Haemophilus* sp., and *Streptococcus* spp., the antimicrobial agents were incorporated into Mueller-Hinton agar supplemented with 5% sheep blood. Tests were performed as described in the *Manual of Clinical Microbiology* (15). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* 25923 were included as controls with each test run.

Antimicrobial susceptibility results for the test strains are shown in Tables 1 and 2. Results with the anaerobic control strains were within acceptable ranges for the agents for which these values have been established (5). Results for the facultative control strains were also within acceptable limits (15).

Penicillin G was active at levels of 2 U/ml against all except six of the strains tested. The reference strain of *B. corporis*, a clinical isolate of *B. melaninogenicus*, and a *Bacteroides* species of oral origin had an MIC of ≥32 U/ml, whereas one *Fusobacterium nucleatum* strain and one *Actinomyces odontolyticus* strain each had an MIC of 8 U/ml, and one *B. ureolyticus* strain had an MIC of 4 U/ml. Thus, almost 98% of all of the recent oral isolates tested were susceptible to 2 U or less of penicillin G, indicating that this antibiotic should continue to pro-

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TABLE 1. Susceptibility of anaerobic isolates to antimicrobial agents

Antimicrobial agent	MIC for[a]: Black-pigmented *Bacteroides*[b] (40)		*Fusobacterium* (13)		Other Gram-negative bacilli[c] (13)		*Veillonella* (8)		Gram-positive cocci (11)		*Eubacterium* (7)	
	Range	90%	Range	90%	Range	90%	Range	90%	Range	90%	Range	90%
Penicillin G	≦0.06–64	0.5	≦0.06–8	0.5	≦0.06–32	4	0.25–0.5	0.5	≦0.06–0.5	0.25	≦0.06–0.5	0.5
Cefadroxil	≦0.06–128	4	0.25–16	8	0.25–64	64	≦0.06–1	0.5	≦0.06–64	16	0.13–64	4
Cephalexin	0.5–32	2	0.5–8	8	0.5–16	16	0.25–1	0.5	0.13–32	8	0.25–64	4
Cephradine	0.25–32	2	0.5–8	8	0.5–32	32	0.13–0.5	0.25	0.5–128	32	0.5–64	16
Cefoperazone	0.13–8	2	≦0.06–8	1	0.25–128	32	0.25–2	2	≦0.06–1	1	≦0.06–2	1
Moxalactam	≦0.06–32	1	0.25–16	16	≦0.06–16	8	≦0.06–2	1	≦0.06–4	2	≦0.06–8	0.5
Sch 29,482	≦0.06–2	0.13	≦0.06–1	1	≦0.06–1	0.5	0.13–0.5	0.25	≦0.06–1	0.5	≦0.06–025	0.25
Clindamycin	≦0.06–0.5	0.13	≦0.06–0.25	0.25	≦0.06–2	1	≦ 0.6–0.25	0.25	≦0.06–0.5	0.5	≦0.06–2	1
Erythromycin	0.13–>128	1	0.5–128	128	≦0.06–2	2	16–64	64	≦0.06–2	2	≦0.06–0.25	0.25
Metronidazole	≦0.06–32	1	≦0.06–0.25	0.25	≦0.06–2	2	0.5–1	1	≦0.06–2	2	≦0.06–64	64
Tetracycline	≦0.06–16	2	≦0.06–16	1	≦0.06–16	1	0.5–2	2	≦0.06–2	1	≦0.06–4	1
Colistin	0.5–>128	>128	0.25–2	1	≦0.06–>128	>128	1–2	1	32–>128	>128	8–>128	>128
Kanamycin	8–>128	>128	0.5–>128	128	0.5–>128	>128	32–>128	64	0.5–>128	128	4–>128	>128
Vancomycin	4–>128	128	16–>128	>128	1–>128	>128	32–>128	>128	0.13–1	1	0.5–2	1

[a] Concentrations are expressed in micrograms per milliliter, except penicillin G, which is expressed in units per milliliter. Numbers in parentheses indicate number of strains tested. 90%, MIC inhibiting 90% of isolates.

[b] Includes *B. melaninogenicus, B. loeschii, B. denticola, B. intermedius, B. corporis, B. asaccharolyticus, B. gingivalis*, and the type strain of *B. macacae*.

[c] Includes *B. oralis, B. ureolyticus*, other *Bacteroides* spp., *Selenomonas* sp., and *Wolinella* sp.

TABLE 2. Susceptibility of microaerophilic and facultative isolates to antimicrobial agents

Antimicrobial agent	MIC for[a]:											
	Capnocytophaga (17)		*Eikenella* and *Haemophilus* (2)[b]		*Actinomyces* (22)[c]		*Arachnia* and *Propionibacterium* (6)[c]		*Lactobacillus* (16)[c]		*Streptococcus* (39)	
	Range	90%	Range	90%	Range	90%	Range	90%	Range	90%	Range	90%
Penicillin G	0.25–1	1	0.25–0.5	0.5	≦0.6–8	1	≦0.6–0.13	0.13	≦0.06–0.5	0.25	≦0.06–0.5	0.25
Cefadroxil	2–>128	128	8–32	32	≦0.06–2	1	0.13–8	8	≦0.06–1	0.5	0.25–32	16
Cephalexin	1–128	64	4–16	16	≦0.06–2	1	0.25–32	32	≦0.06–2	0.5	0.5–32	16
Cephradine	2–>128	128	8–16	16	0.25–8	4	0.25–64	64	≦0.06–1	1	0.25–32	16
Cefoperazone	0.25–32	8	≦0.06–0.13	0.13	0.13–4	4	0.13–8	8	≦0.06–1	1	≦0.06–4	2
Moxalactam	0.13–16	4	≦0.06	≦0.06	≦0.06–8	4	≦0.06–8	8	0.13–1	0.5	≦0.06–32	8
Sch 29,482	0.25–2	1	0.25–1	1	≦0.06–1	0.5	≦0.06–4	4	≦0.06–0.25	0.25	≦0.06–2	1
Clindamycin	≦0.06–0.13	0.13	16–32	32	≦0.06–4	1	≦0.06–16	16	≦0.06–2	1	≦0.06–128	0.13
Erythromycin	0.13–4	2	1	1	≦0.06–1	0.25	≦0.06–2	2	≦0.06–1	0.5	≦0.06–>128	0.13
Metronidazole	1–32	16	128–>128	>128	0.25–>128	>128	0.5–>128	>128	0.5–>128	>128	128–>128	>128
Tetracycline	0.25–2	2	1	1	0.25–64	4	0.13–2	2	≦0.06–16	1	0.5–128	64
Colistin	128–>128	>128	0.5–1	1	16–>128	>128	128–>128	>128	8–>128	>128	128–>128	>128
Kanamycin	128–>128	>128	1–2	2	8–>128	128	16–>128	>128	2–128	128	0.5–>128	>128
Vancomycin	0.5–64	32	16–128	128	0.5–8	1	0.25–64	64	0.13–2	1	0.5–2	2

[a] Concentrations are expressed in micrograms per milliliter, except penicillin G, which is expressed in units per milliliter. Numbers in parentheses indicate number of strains tested. 90%, MIC inhibiting 90% of isolates.

[b] One strain each of *E. corrodens* and *H. aphrophilus*.

[c] A few strains grew under anaerobic conditions only.

vide adequate therapy for a variety of oral infections.

β-Lactamase production was not determined for these strains. However, in contrast to reports of increasing penicillin resistance and β-lactamase production among isolates from other types of patients where history of antibiotic usage was not given (2, 4), there is a very low incidence of resistance and β-lactamase production in recent studies (3, 11). When isolates are taken from patients without history of recent antibiotic usage (11) or from those not recently hospitalized (3), the incidence appears to be approximately 10%.

The activities of the oral cephalosporins cefadroxil, cephalexin, and cephradine, as well as tetracycline, were variable among the different groups of bacteria, indicating that their therapeutic effectiveness is unpredictable on the basis of these data.

The newer cephalosporins moxalactam and Sch 29,482 were active at achievable levels (16 to 32 μg/ml) against all strains tested. Only one *B. ureolyticus* strain was resistant to cefoperazone. This strain was relatively resistant to penicillin G and was also resistant to the oral cephalosporins but susceptible to tetracycline.

Erythromycin was active against 90% or more of the strains with the exception of *Fusobacterium* spp. and *Veillonella* spp. Clindamycin was active against all but the *Eikenella* sp., *Haemophilus* sp., and one *Propionibacterium* strain. Metronidazole was active against more than 90% of the anaerobic bacteria and *Capnocytophaga* spp. It was not active against most of the other microaerophilic and facultative bacteria.

Results with some of the antibiotics used in selective media for anaerobes and other oral bacteria indicated that at concentrations in common use (10 μg/ml for colistin, 75 or 100 μg/ml for kanamycin, and 7.5 μg/ml for vancomycin), one could anticipate some inhibition of desirable bacteria or lack of inhibition of others. Colistin is often used to allow growth of gram-positive bacteria and inhibit growth of gram-negative bacteria. Our data show that only 2% of the gram-positive strains were inhibited by 8 μg/ml. However, 34% of the gram-negative strains were not inhibited by this concentration of colistin.

The combination of kanamycin and vancomycin has been recommended for selective isolation of the black-pigmented and other *Bacteroides* species (9). Our present data show that one strain each of *B. corporis*, *B. oralis*, and *B. ureolyticus* and two *Bacteroides* species were inhibited by ≦64 μg of kanamycin per ml. Vancomycin at 4 μg/ml was inhibitory to three of five *B. asaccharolyticus* strains, one of two *B. gingivalis* strains, and the *B. macacae* strain. The second *B. gingivalis* strain was inhibited by 8 μg/ml. It appears that incorporation of vancomycin (7.5 μg/ml) in media would be detrimental to selective isolation of asaccharolytic black-pigmented *Bacteroides* species and possibly to animal strains as well. These results indicate that there is a need for further investigation of agents suitable for selective isolation of *Bacteroides* species, particularly for the black-pigmented species.

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