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Penicillin Resistance in the Subgingival Microbiota Associated with Adult Periodontitis

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In this investigation, the penicillin-resistant and beta-lactamase-producing subgingival microbiota associated with adult periodontitis was identified, and the impact of a recent exposure to penicillin on the recovery of resistant organisms from this microbiota was assessed. Subjects with adult periodontitis were examined clinically and microbiologically. Twenty-one subjects had a documented history of penicillin therapy within the previous 6 months whereas an additional 21 subjects had no history of antibiotic use within 1 year. Subgingival plaque samples were cultured anaerobically on nonselective and penicillin-containing elective media. MICs and beta-lactamase production were determined for the isolates from the elective medium. The penicillin-resistant microbiota consisted primarily of gram-negative organisms, including *Bacteroides*, *Veillonella*, *Haemophilus*, *Eikenella*, and *Capnocytophaga* species. The prevalence ($P < 0.05$) and proportions ($P < 0.005$) of both penicillin-resistant pigmented *Bacteroides* and *Veillonella* species were significantly greater in subjects with recent penicillin exposure. Of the penicillin-resistant genera identified, beta-lactamase production was detected in species of pigmented *Bacteroides*, *Capnocytophaga*, and *Streptococcus*. The prevalence of beta-lactamase-producing *Bacteroides* species was significantly greater in subjects with recent penicillin exposure ($P < 0.05$). Of the antibiotics examined, no single agent was uniformly effective against all of the penicillin-resistant strains, but metronidazole and clindamycin were active against all of the penicillin-resistant pigmented *Bacteroides* strains.

Penicillin has long been considered the drug of choice in the treatment of odontogenic infections (11, 27). However, reports of infections resistant clinically to penicillin therapy (1, 4, 13, 41, 44), as well as reports of penicillin resistance among species associated with these infections (8, 23), have recently caused concern about the empiric use of this antibiotic.

Although a variety of bacterial genera have previously been associated with odontogenic infections, the predominant bacteria recovered include *Bacteroides*, *Fusobacterium*, *Peptococcus*, and *Peptostreptococcus* species (3, 6, 7, 41, 42, 46). The involvement of *Bacteroides* species in odontogenic infections is of particular interest, as investigations of abscess formation in animal models indicate a key role of these species in the production and transmissibility of experimental infections (21, 22, 32). Increasing patterns of penicillin resistance have been reported among species other than *Bacteroides fragilis* in surveys from hospital clincal laboratories (8, 23), and microbiological examinations of infections resistant to penicillin therapy have frequently yielded beta-lactamase-producing *Bacteroides* species (4, 13, 41). The use of beta-lactam antibiotics has been associated with the emergence of beta-lactamase-producing *Bacteroides* species in the salivary and oropharyngeal microbiota (5, 14).

Many of the bacterial species implicated in odontogenic infections are routinely isolated from the subgingival microbiota and, as in the case of the *Bacteroides* species, are found in increased numbers in the subgingival microbiota of individuals with adult periodontitis (31, 38, 45). The present investigation was undertaken to characterize the penicillin-resistant and beta-lactamase-producing subgingival microbiota from individuals with adult periodontitis, to examine the impact of a recent exposure to penicillin on the recovery of these species, and to determine the susceptibility of these microorganisms to antibiotic agents typically used as alternatives to penicillin in the treatment of odontogenic infections.

(This research was conducted by S. A. Kinder in partial fulfillment of the requirements for the M. Dent. Sc. degree from the University of Connecticut, Farmington, 1985. A preliminary report of this work has been presented [S. A. Kinder and K. S. Kornman, Abstr. 63rd Gen. Session Int. Assn. Dent. Res., 1985, abstr. no. 1622, p. 355].)

MATERIALS AND METHODS

Subjects. A total of 42 subjects, 26 males and 16 females (mean age of 49 years), were selected from the university outpatient dental clinics. The subjects selected were in generally good health; individuals with diabetes, autoimmune disorders, or other conditions potentially influencing their periodontal condition were not included. All subjects had adult periodontitis, based on the criteria of a minimum age of 30 years and the presence of periodontal lesions with probing depths of ≥5 mm in addition to crestal alveolar bone loss of ≥4 mm as measured on recent bitewing radiographs. The majority of subjects were untreated periodontally, and none had received scaling or root planing within 2 months prior to participation in the study. One half of the subjects had not received any antibiotics within the previous year; these subjects were designated the Pen(−) group. For the remaining subjects, the Pen(+) group, a course of a non-penicillinase-resistant penicillin (e.g., penicillin V or ampicillin) had been prescribed within the previous 1 to 6 months. The course of penicillin consisted of a minimum dosage of 1 g per day for

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an average of 8 days and was confirmed through consultation with the subject's physician or dentist. Subjects with a history of chronic antibiotic use were excluded, and only one subject had received an additional separate course of penicillin within 1 year prior to participating in the study. Three subjects had received additional antibiotics within the previous 6 months: one subject had received tetracycline and bactrim, one had received erythromycin, and one had received metronidazole.

Clinical procedures. Clinical and microbiological examinations (see below) were carried out on two sites in the premolar/molar region per subject, with two exceptions: one Pen(+) subject had only one site considered appropriate based on selection criteria and a microbiological sample was lost for one Pen(−) subject. Clinical examinations consisted of a plaque index (30), a modified gingival index (20), and probing depth and clinical attachment level measurements. The scoring protocol for the gingival index was modified to accommodate the sampling procedure; a score of 2 was used to indicate bleeding after paper point placement (see microbiological procedures). The other scoring remained unchanged.

Microbiological procedures. Microbiological sampling was carried out after careful debridement of the supragingival plaque, but before probing. Subgingival plaque samples were obtained and processed according to the method of Kornman et al. (17). Briefly, three sterile paper points were placed to the depth of the periodontal pocket for 10 s. The paper points were removed and immediately placed in a vial of sterile reduced transport fluid (35) and transported to a Coy anaerobic chamber for processing (Coy Laboratory Products, Ann Arbor, Mich.). The plaque samples were dispersed, diluted in sterile reduced transport fluid, and plated onto nonselective and elective media with an automatic diluting and plating device (Spiral Systems, Bethesda, Md.). Enriched tryptic soy agar (ETSA) was used as a nonselective medium (36), while ETSA supplemented with pencillin G (pen-ETSA) was used as an elective medium for the recovery of penicillin-resistant organisms. The penicillin concentration used in the pen-ESTA medium was titrated to achieve a maximum level of 2 μg of penicillin G activity per ml (data not shown). After 5 to 7 days of incubation in the anaerobic chamber at 37°C, the total number of CFU was determined on both media. Colonies presumed to be pigmented *Bacteroides* strains on the basis of pigmentation or fluorescence under UV illumination were subcultured from the ETSA medium. From the pen-ETSA medium all presumed pigmented *Bacteroides* strains, as well as representative colonies of all other morphological types, were subcultured onto the ETSA medium.

Pure cultures of the strains isolated from the pen-ETSA medium were characterized by MICs of selected antibiotics, as well as by the presence or absence of beta-lactamase production (see below). For the purposes of this investigation, penicillin resistance was defined by an MIC of penicillin G of ≥4 μg/ml or by the detection of beta-lactamase production. All isolates from the ETSA medium, as well as the penicillin-resistant isolates from the pen-ETSA medium, were identified to the genus and species level according to current taxonomic schemes. The identification was based on colonial and cellular morphology, biochemical reactions, fermentation patterns, use of chromogenic substrates (An-Ident; API, Plainville, N.Y.), and gas-liquid chromatographic analysis of metabolic end products (9, 16, 18). Selected strains were additionally characterized by SDS-polyacrylamide gel electrophoresis of precipitated and soluble proteins by the method of Pearlman et al. (26) with modifications (S. A. Kinder, K. S. Kornman, and S. C. Holt, manuscript in preparation). Strains previously designated as *Actinobacillus actinomycetemcomitans* are included in the *Haemophilus* genus according to the proposal of Potts et al. (28). The pigmented *Bacteroides* species include *Bacteroides intermedius*, *Bacteroides melaninogenicus*, *Bacteroides denticola*, *Bacteroides loescheii*, and *Bacteroides gingivalis*.

MIC determinations. The MICs of penicillin G, ampicillin, metronidazole, tetracycline (Sigma Chemical Co., St. Louis, Mo.), and clindamycin (The Upjohn Co., Kalamazoo, Mich.) were determined for all isolates from the pen-ETSA medium. Antibiotic dilutions were prepared in sterile distilled water and stored at −70°C until used. MIC determinations were carried out according to the agar dilution technique recommended for anaerobic species (24) with the following modifications: the Wilkins-Chalgren test medium was supplemented with 3% (vol/vol) sheep blood (Colorado Serum, Colorado Springs, Colo.), 0.05% (wt/vol) sodium formate, and 0.02% (wt/vol) sodium fumarate. Bacterial strains to be tested were suspended in the recommended thioglycolate broth to a McFarland standard of 0.5 immediately prior to inoculation. All MIC plates were inoculated in the Coy anaerobic chamber with a replicating device (Repliplate; Cathra International, Inc.). Control cultures included in each test run were *B. fragilis* (ATCC 25285) and *Bacteroides thetaiotaomicron* (ATCC 29741); the MICs for these strains were within the previously defined range of acceptable MICs (24).

Beta-lactamase detection. Beta-lactamase production was determined for all pigmented *Bacteroides* species, as well as for all strains isolated from the pen-ETSA medium with an MIC of penicillin G of ≥2 μg/ml. A chromogenic cephalosporin technique was used (with Cefinase disks; BBL Microbiology Systems, Cockeysville, Md.), as this method has been shown to be the most reliable for anaerobic species (E. Burkholder, S. Hansen, C. Benton, P. Freedy, T. Williams, S. Marubio, R. Yogeu, and K. Eisenach, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C303, p. 362). Positive and negative beta-lactamase controls consisted of pigmented *Bacteroides* strains VPI 9331 and VPI 8944, respectively (29).

Statistical analysis. Data analysis was carried out with the *Statistical Package for the Social Sciences* (version 9.1; SPSS Inc., Chicago, Ill.). Differences between the Pen(−) and Pen(+) groups were examined with the Student's *t* test for parametric data and the Mann-Whitney U-test or chi-square test, with Yate's correction, for nonparametric data. The focus of the statistical analysis was to examine the impact on the recovery of resistant organisms of a history of recent penicillin administration. This is a subject variable, and the analysis was therefore based on subject means (n = 42), with the exception of the analyses of plaque and gingival indices, which were based on data from sample sites (n = 82). Due to technical difficulties, quantitative data was not available on the single Pen(+) sample from which penicillin-resistant *Streptococcus* species were recovered. Thus, analysis of the proportional data was not possible for this group of microorganisms.

RESULTS

The Pen(−) and Pen(+) groups were very uniform in their clinical profile (Table 1), and these data are consistent with a diagnosis of adult periodontitis. The two study groups were also very similar in the microbiological recovery from

TABLE 1. Clinical profile of subject populations

Subject group (n = 21)	Age (yr)	Plaque index	Gingival index	Probing depth (mm)	Attachment loss (mm)
Pen (−)	52.6 ± 12.0[a]	1.5 ± 1.0	1.9 ± 0.4	6.0 ± 1.0	6.7 ± 1.3
Pen (+)	45.5 ± 13.1	1.3 ± 0.8	1.9 ± 0.5	6.2 ± 1.3	6.9 ± 1.1

[a] Mean ± standard deviation. No significant differences were found between the groups for any characteristic. All characteristics except for age are based on two sample sites per subject.

ETSA; no significant differences in the total number of CFU or the number and distribution of isolated pigmented *Bacteroides* species were found (Table 2).

The penicillin-resistant strains recovered from the Pen(−) group represented 1.7% of the total cultivable subgingival microbiota (Table 3). In contrast, penicillin-resistant strains represented 3.8% of the total cultivable microbiota in the Pen(+) group, and this represented a significant difference between the two study groups ($P < 0.05$). The increased recovery of resistant microorganisms in the Pen(+) group was accounted for primarily by a greater recovery of the anaerobic ($P < 0.005$) and gram-negative ($P < 0.005$) species. The distribution of penicillin-resistant species in the two study groups was the same, and the majority of resistant species were found to be members of the *Bacteroides*, *Veillonella*, *Eikenella*, *Capnocytophaga*, and *Haemophilus* genera (Table 3). Significantly greater percentages of resistant pigmented *Bacteroides* species ($P < 0.005$), *B. intermedius* ($P < 0.05$), the *B. melaninogenicus* and *B. denticola* group ($P < 0.05$), the *Veillonella* species ($P < 0.005$), and *Veillonella dispar* ($P < 0.005$) were isolated from the Pen(+) subjects (Table 3). Virtually all subjects were found to harbor penicillin-resistant species (Fig. 1A). However, the prevalence of most resistant species was greater in the Pen(+) group, as compared with the Pen(−) group (Fig. 1), with a significantly greater prevalance in the Pen(+) group of penicillin-resistant pigmented *Bacteroides* species, *B. intermedius*, and *Veillonella* species ($P < 0.05$; Fig. 1B).

Beta-lactamase-producing species represented 0.7 and 1.3% of the total cultivable subgingival microbiota of the Pen(−) and Pen(+) groups, respectively (Table 3). Of the penicillin-resistant microorganisms identified, all pigmented *Bacteroides* species with an MIC of penicillin G of ≥1 μg/ml, as well as all resistant *Capnocytophaga* and *Streptococcus* species, were found to produce beta-lactamase. The prevalence of beta-lactamase-producing subgingival microorganisms is shown in Fig. 1A. Although 76% of the Pen(+) subjects were found to harbor beta-lactamase-producing microorganisms, compared with 48% in the Pen(−) group, this difference was not statistically significant. In contrast, the prevalence of pigmented *Bacteroides* species (Fig. 1B), all of which were found to produce beta-lactamase, was significant in the Pen(+) group ($P < 0.05$).

The in vitro susceptibilities of the predominant penicillin-resistant strains are shown in Table 4. Since no differences were observed in the susceptibility patterns of the isolates recovered from either group, the data were combined. The strains reported here were initially selected on the basis of their resistance to penicillin, and this is reflected in the MIC_{50} (the MIC for 50% of the strains) and the MIC_{90} of penicillin G for all strains. High levels of penicillin resistance occurred among *Haemophilus* and *Streptococcus* species, for which the MIC_{90} of penicillin G was ≥64 μg/ml. Ampicillin demonstrated comparable or slightly greater activity against most species. For example, for the 32 isolates of *B. intermedius*, the MIC_{50} of ampicillin was 8 μg/ml, compared with an MIC_{50} of penicillin G of 16 μg/ml for the same strains. Tetracycline resistance was evident in the pigmented *Bacteroides* species, with the MIC_{90} ranging from 8 to 16 μg/ml. In contrast, the pigmented *Bacteroides* strains were uniformly susceptible to both metronidazole (MIC_{90} of 0.5 to 2 μg/ml) and clindamycin (MIC_{90} all ≤0.25 μg/ml). All of the facultative isolates, i.e., the *Haemophilus*, *Capnocytophaga*, and *Streptococcus* species and *Eikenella corrodens*, were resistant to metronidazole (MIC_{90} of 16 to ≥128 μg/ml). Whereas clindamycin inhibited the growth of most isolates, resistance to this antibiotic was seen in the nonpigmented *Bacteroides* species, *Bacteroides gracilis* (for which the MIC_{90} was 8 μg/ml), *E. corrodens* (for which the MIC_{90} was ≥128 μg/ml), and in the *Haemophilus* strains (for which the MIC_{90} was 64 μg/ml).

DISCUSSION

Penicillin resistance among microorganisms found in the subgingival microbiota is of significance in light of the routine use of penicillin in the treatment of infections involv-

TABLE 2. Microbiological recovery on nonselective medium

Subject group	% (range) total CFU of strain or strain group[a]						
	Pigmented *Bacteroides* species	*B. intermedius*	*B. melaninogenicus*/ *B. denticola*	*B. loescheii*	*B. gingivalis*	Unspeciated pigmented *Bacteroides* species[b]	Total (range) CFU (10^6)
Pen (−)	12.8 (3.0–23.5)	9.5 (ND[c]–23.3)	0.5 (ND–3.4)	<0.1 (ND–0.8)	1.0 (ND–12.3)	1.7 (ND–9.8)	32.7 (4.4–143.8)
Pen (+)	10.6 (ND–54.5)	6.0 (ND–16.7)	0.6 (ND–8.2)	0.3 (ND–2.7)	2.5 (ND–48.5)	1.2 (ND–10.3)	24.2 (0.9–65.8)

[a] Figures represent mean percentages of total CFU for strains found in all subjects in a group. No significant differences were found between the groups for any one strain. Mann-Whitney U-test (two-tailed).

[b] Strains not recovered on subculture.

[c] ND, Not detected.

TABLE 3. Penicillin-resistant subgingival microbiota

Subject group	Mean % (range) of total CFU by subject of penicillin-resistant strain or strain group[a]															
	Anaerobic microorganisms							Facultative microorganisms			Totals					
	B. intermedius	*B. melaninogenicus/B. denticola*	*B. loescheii*	*B. gracilis*	Total pigmented *Bacteroides* species	Total *Bacteroides* species	*Veillonella parvula/Veillonella atypica*	*Veillonella dispar*	Total *Veillonella* species	*E. corrodens*	Total *Haemophilus* species	Total *Capnocytophaga* species	Anaerobic species	Gram-negative species	Beta-lactamase-producing species[b]	Penicillin-resistant species
Pen (−)	0.2 (ND[c]–4.9)	0.1 (ND–1.3)	<0.1 (ND–0.2)	0.2 (ND–1.5)	0.3 (ND–4.9)	0.5 (ND–4.9)	<0.1 (ND–0.5)	<0.1 (ND–0.2)	<0.1 (ND–0.5)	0.2 (ND–2.0)	0.2 (ND–2.5)	<0.1 (ND–0.3)	0.7 (ND–4.9)	1.2 (ND–5.8)	0.7 (ND–4.9)	1.7 (ND–9.7)
Pen (+)	0.6[d] (ND–7.4)	0.6[d] (ND–7.3)	<0.1 (ND–0.6)	0.4 (ND–0.6)	1.3[e] (ND–7.4)	1.7[e] (ND–7.6)	0.2 (ND–1.6)	0.4[e] (ND–2.4)	0.6[e] (ND–3.3)	0.8 (ND–9.4)	<0.1 (ND–0.4)	<0.1 (ND–0.7)	2.6[e] (0.1–10.7)	3.7[e] (0.2–19.9)	1.3 (ND–7.4)	3.8[d] (0.2–19.9)

[a] Penicillin resistance defined as an MIC of ≥4 μg/ml or by beta-lactamase production.
[b] Includes pigmented *Bacteroides*, *Capnocytophaga*, and *Streptococcus* species.
[c] ND, Not detected.
[d] $P < 0.05$, as determined by the Mann-Whitney U test (one-tailed). The one-tailed test was used since previous investigations have documented greater recoveries of penicillin-resistant species in association with recent penicillin administration.
[e] $P < 0.005$, Mann-Whitney U test (one-tailed).

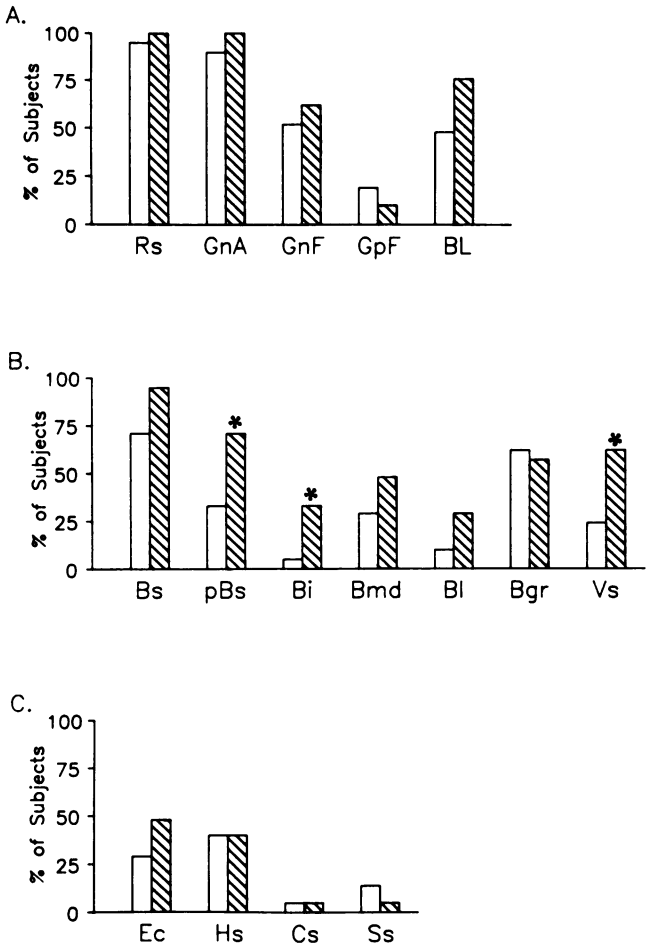

FIG. 1. Prevalence of the penicillin-resistant microbiota from the Pen(+) (▨), and Pen(−) (□) groups. (A) Prevalence of general groups of penicillin-resistant microorganisms; (B) prevalence of predominant penicillin-resistant anaerobic species; (C) prevalence of predominant penicillin-resistant facultative species. Abbreviations: Rs, total resistant microorganisms; GnA, resistant gram-negative anaerobic species; GnF, resistant gram-negative facultative species; GpF, resistant gram-positive facultative species; BL, beta-lactamase-producing species; Bs, total *Bacteroides* species; pBs, total pigmented *Bacteroides* species; Bi, *B. intermedius*; Bmd, *B. melaninogenicus/B. denticola*; Bl, *B. loescheii*; Bgr, *B. gracilis*; Vs, *Veillonella* species; Ec, *E. corrodens*; Hs, *Haemophilus* species; Cs, *Capnocytophaga* species; Ss, *Streptococcus* species. *, $P < 0.05$, chi-square test.

ing these species (11, 27). This paper describes the first report of a systematic survey of subjects with adult periodontitis for subgingival penicillin-resistant microorganisms based on in vitro susceptibility testing. Previous reports of the susceptibility of isolates from combinations of periodontally healthy and diseased subjects found penicillin to be active against most microbial species found in the subgingival region (2, 34). We found that a small proportion of the total subgingival microorganisms associated with adult periodontitis (less than 3%) were resistant to penicillin. The predominant penicillin-resistant subgingival isolates recovered in this investigation consisted of *Bacteroides*, *Veillonella*, *Haemophilus*, *Eikenella*, *Capnocytophaga*, and *Streptococcus* species. This finding is generally consistent with previous susceptibility studies (12, 19, 33, 34, 37, 43).

Previous studies have also reported that the beta-

TABLE 4. In vitro susceptibility of penicillin-resistant subgingival isolates to selected antibiotics

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
B. gracilis (52)	Penicillin G	4->128	4	32
	Ampicillin	≤0.25->128	1	4
	Tetracycline	≤0.25-32	1	4
	Metronidazole	≤0.25-16	0.5	4
	Clindamycin	≤0.25-64	2	8
Veillonella parvula/ *Veillonella atypica* (19)	Penicillin G	4-32	8	16
	Ampicillin	≤0.25-8	1	2
	Tetracycline	≤0.25-2	1	2
	Metronidazole	≤0.25-4	1	2
	Clindamycin	≤0.25-2	≤0.25	2
Veillonella dispar (19)	Penicillin G	4->128	8	32
	Ampicillin	≤0.25->128	8	64
	Tetracycline	≤0.25-64	2	4
	Metronidazole	≤0.25-8	2	4
	Clindamycin	≤0.25-8	≤0.25	2
B. intermedius (32)	Penicillin G	2-64	16	16
	Ampicillin	0.5-64	8	16
	Tetracycline	≤0.25-16	1	8
	Metronidazole	≤0.25-0.5	≤0.25	0.5
	Clindamycin	≤0.25-2	≤0.25	≤0.25
B. melaninogenicus/ *B. denticola* (48)	Penicillin G	1-64	8	32
	Ampicillin	0.5-64	8	32
	Tetracycline	≤0.25-64	0.5	16
	Metronidazole	≤0.25-4	≤0.25	2
	Clindamycin	≤0.25-1	≤0.25	≤0.25
B. loescheii (24)	Penicillin G	2-64	16	32
	Ampicillin	1-64	16	32
	Tetracycline	≤0.25-64	0.5	16
	Metronidazole	≤0.25-2	0.5	1
	Clindamycin	≤0.25	≤0.25	≤0.25
Haemophilus species (11)	Penicillin G	4->128	8	>128
	Ampicillin	≤0.25->128	8	32
	Tetracycline	0.5-4	2	4
	Metronidazole	2->128	4	32
	Clindamycin	4-128	8	64
Capnocytophaga species (7)	Penicillin G	4-16	8	16
	Ampicillin	≤0.25-8	4	8
	Tetracycline	≤0.25-2	1	1
	Metronidazole	2-16	8	16
	Clindamycin	≤0.25	≤0.25	≤0.25
E. corrodens (25)	Penicillin G	4->128	4	8
	Ampicillin	1->128	2	4
	Tetracycline	0.5-4	1	4
	Metronidazole	32->128	128	>128
	Clindamycin	8->128	128	>128
Streptococcus species (6)	Penicillin G	32->128	>128	>128
	Ampicillin	16->128	32	64
	Tetracycline	0.5-4	2	4
	Metronidazole	>128	>128	>128
	Clindamycin	≤0.25-1	0.5	1

lactamase-producing microorganisms recovered from the periodontal microbiota belong primarily to the pigmented *Bacteroides* species (40). In this investigation, approximately one-third of the penicillin-resistant subgingival microorganisms isolated produced beta-lactamase, and the majority of these strains were pigmented *Bacteroides* species. There was a significantly greater proportion of beta-lactamase-producing pigmented *Bacteroides* species recovered from individuals who had a history of recent penicillin therapy ($P < 0.005$). In contrast to Valdés et al. (40), who did not report the presence of beta-lactamase-producing *B. intermedius* strains from the subgingival microbiota, *B. intermedius* did represent a significant proportion of the beta-lactamase-producing strains recovered in the present study (Table 3). The reason for this difference is unclear, although it may be due to the use of different media and a different technique of beta-lactamase detection (Valdés et al. used the microiodometric method) (40). Beta-lactamase-producing strains of *B. intermedius* isolated from the subgingival microbiota have been reported elsewhere (C. B. Walker, J. D. Pappas, and M. B. Erlich, Abstr. 62nd Gen. Session Int. Assn. Dent. Res., 1984, abstr. no. 466, p. 222). *B. gingivalis* accounted for 15% of the pigmented *Bacteroides* recovered on the pen-ETSA medium (Table 2), but interestingly, no beta-lactamase-producing or penicillin-resistant *B. gingivalis* strains were detected. The reason for the lack of penicillin resistance in *B. gingivalis*, in contrast to the other pigmented *Bacteroides* species, is unclear.

In addition to the *Bacteroides* species, the other beta-lactamase-producing bacteria recovered in this study included *Capnocytophaga* and *Streptococcus* species. This is a significant finding, since beta-lactamase production in the genus *Capnocytophaga* had not previously been reported. Although previous studies have reported that oral *Veillonella* species produce beta-lactamase (40), we were not able to detect production of the enzyme in the penicillin-resistant *Veillonella* species recovered in this investigation. Furthermore, whereas penicillin has often been reported effective against *Veillonella* species (2, 34), penicillin resistance has also been reported in this genus (12, 15, 40).

Although the actual numbers of penicillin-resistant and beta-lactamase-producing species recovered in this investigation were small, one would expect these species to proliferate under the selective pressure of penicillin administration. Thus, in assessing the impact of the presence of these microorganisms in the subgingival microbiota, the prevalence of resistant microorganisms may be of more importance that the actual numbers of microorganisms present. In this investigation, both the prevalence and the proportions of penicillin-resistant and beta-lactamase-producing pigmented *Bacteroides* species were significantly greater in subjects with a recent history of penicillin administration ($P < 0.05$ and $P < 0.005$, respectively). In light of the frequent association of *Bacteroides* species with odontogenic infections, these results suggest that in patients with adult periodontitis and odontogenic infections caused by periodontal microorganisms, those individuals with a history of recent penicillin administration should be considered at greater risk for infections that will not respond to therapy with penicillin.

Beta-lactamase production has been found to be mediated in certain microbial pathogens by transposable genetic elements (e.g., plasmids) and is associated with the rapid and widespread dissemination of penicillin resistance in species such as *Neisseria gonorrhoeae* and *Haemophilus influenzae* (10, 25, 39). The genetic basis of beta-lactamase production in the species described in this investigation has not been reported; if mediated by transposable genetic elements, however, penicillin resistance in subgingival pathogens may become a rapidly increasing clinical problem.

The susceptibility of the penicillin-resistant subgingival

microorganisms, particularly the *Bacteroides* species, to other antibiotics was of interest because of reports of odontogenic infections that have not responded clinically to penicillin therapy. Of the antibiotics commonly recommended for the treatment of odontogenic infections (Table 4), we found that none were uniformly active against all of the penicillin-resistant strains recovered from the subgingival microbiota. Tetracycline was more active against the majority of these strains compared with the other antibiotics, but significant levels of resistance were seen in the pigmented *Bacteroides* strains. Both metronidazole and clindamycin were effective in inhibiting the growth of many of these strains, including the pigmented *Bacteroides* species. Antimicrobial agents not examined in this investigation include newly developed combinations of beta-lactam compounds with beta-lactamase inhibitors (25). These agents may be beneficial in the treatment of penicillin-resistant odontogenic infections, and investigation of the susceptibility of common pathogens to these combined agents is warranted.

In summary, in this investigation a recent systemic penicillin exposure in subjects with adult periodontitis was associated with a greater recovery of penicillin-resistant pigmented *Bacteroides* and *Veillonella* species from the subgingival microbiota. Beta-lactamase production was detected in pigmented *Bacteroides*, *Capnocytophaga*, and *Streptococcus* species. In vitro susceptibility data for the antibiotics investigated here indicated that both metronidazole and clindamycin were active against all of the penicillin-resistant pigmented *Bacteroides* strains and against many other penicillin-resistant subgingival microorganisms.

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LITERATURE CITED

1. **Bahn, S. L., B. Ciola, and A. G. Segal.** 1981. Penicillin-resistant *Bacteroides melaninogenicus* infection of the mandible. J. Oral Surg. **39**:221–223.
2. **Baker, P. J., J. Slots, R. J. Genco, and R. T. Evans.** 1983. Minimal inhibitory concentrations of various antimicrobial agents for human oral anaerobic bacteria. Antimicrob. Agents Chemother. **24**:420–424.
3. **Bartlett, J. G., and P. O'Keefe.** 1979. The bacteriology of perimandibular space infections. J. Oral Surg. **37**:407–409.
4. **Brook, I.** 1984. Beta-lactamase-producing bacteria recovered after clinical failures with various penicillin therapy. Arch. Otolaryngol. **110**:228–231.
5. **Brook, I., and A. E. Gober.** 1984. Emergence of beta-lactamase producing aerobic and anaerobic bacteria in the oropharynx of children following penicillin chemotherapy. Clin. Pediatr. **23**:338–341.
6. **Brook, I., S. Grimm, and R. B. Kielich.** 1981. Bacteriology of acute periapical abscesses in children. J. Endod. **7**:378–380.
7. **Chow, A. W., S. M. Roser, and F. A. Brady.** 1978. Orofacial odontogenic infections. Ann. Intern. Med. **88**:392–402.
8. **Edson, R. S., J. E. Rosenblatt, D. T. Lee, and E. A. McVey.** 1982. Recent experience with antimicrobial susceptibility of anaerobic bacteria: increasing resistance to penicillin. Mayo Clin. Proc. **57**:737–741.
9. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci, p. 154–175. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
10. **Genco, C. A., J. S. Knapp, and V. Clark.** 1984. Conjugation of plasmids of *Neisseria gonorrhoeae* to other *Neisseria* species: potential reservoirs for the beta-lactamase plasmid. J. Infect. Dis. **150**:397–401.
11. **Goodman, A. D., and M. G. Newman.** 1984. Drugs of choice, p. 39–57. *In* M. G. Newman and A. D. Goodman (ed.), Guide to antibiotic use in dental practice. Quintessence Publishing Co., Inc., Chicago.
12. **Hanson, C. W., and W. J. Martin.** 1980. Antimicrobial susceptibility of anaerobic bacteria isolated from clinical specimens using an agar dilution procedure. Curr. Microbiol. **3**:349–353.
13. **Heimdahl, A., L. von Konow, and C. E. Nord.** 1980. Isolation of beta-lactamase-producing *Bacteroides* strains associated with clinical failures with penicillin treatment of human orofacial infections. Arch. Oral Biol. **25**:689–692.
14. **Heimdahl, A., L. von Konow, and C. E. Nord.** 1981. Beta-lactamase-producing *Bacteroides* species in the oral cavity in relation to penicillin therapy. J. Antimicrob. Chemother. **8**:225–229.
15. **Heimdahl, A., L. von Konow, T. Satoh, and C. E. Nord.** 1985. Clinical appearance of orofacial infections of odontogenic origin in relation to microbiological findings. J. Clin. Microbiol. **22**:299–302.
16. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe Laboratory Manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
17. **Kornman, K. S., S. C. Holt, and P. B. Robertson.** 1981. The microbiology of ligature-induced periodontitis in the cynomolgus monkey. J. Periodontal Res. **16**:363–371.
18. **Krieg, N. R., and J. G. Holt (ed.).** 1984. Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
19. **Laatsch, L. J., P. R. Hohenfeldt, and W. L. Kos.** 1982. Antibiotic susceptibility of black-pigmented *Bacteroides* isolates from the human oral cavity. Antimicrob. Agents Chemother. **22**:698–700.
20. **Löe, H., and J. Silness.** 1963. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol. Scand. **21**:533–551.
21. **MacDonald, J. B., S. S. Socransky, and R. J. Gibbons.** 1963. Aspects of the pathogenesis of mixed anaerobic infections of mucous membranes. J. Dent. Res. **42**(Suppl):529–544.
22. **Mayrand, D., and B. C. McBride.** 1980. Ecological relationships of bacteria involved in a simple, mixed anaerobic infection. Infect. Immun. **27**:44–50.
23. **Murray, P. R., and J. E. Rosenblatt.** 1977. Penicillin resistance and penicillinase production in clinical isolates of *Bacteroides melaninogenicus*. Antimicrob. Agents Chemother. **11**:605–608.
24. **National Committee for Clinical Laboratory Standards.** 1982. Tentative standard reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria, vol. 2, no. 3, p. 70–101. National Committee for Clinical Laboratory Standards, Villanova, Pa.
25. **Neu, H. C.** 1983. The emergence of bacterial resistance and its influence on empiric therapy. Rev. Infect. Dis. **5**(Suppl. 1): S9–S20.
26. **Pearlman, E., N. C. Engleberg, and B. I. Eisenstein.** 1985. Identification of protein antigens of *Legionella pneumophila* serogroup 1. Infect. Immun. **47**:74–79.
27. **Peterson, L. J.** 1981. Principles of antibiotic therapy, p. 133–172. *In* R. G. Topazian and M. H. Goldberg (ed.), Management of infections of the oral and maxillofacial regions. The W. B. Saunders Co., Philadelphia.
28. **Potts, T. V., J. J. Zambon, and R. J. Genco.** 1985. Reassignment of *Actinobacillus actinomycetemcomitans* to the genus *Haemophilus* as *Haemophilus actinomycetemcomitans* comb. nov. Int. J. Syst. Bacteriol. **35**:337–341.
29. **Salyers, A. A., J. Wong, and T. D. Wilkins.** 1977. Beta-lactamase activity in strains of *Bacteroides melaninogenicus* and *Bacteroides oralis*. Antimicrob. Agents Chemother. **11**:142–146.
30. **Silness, J., and H. Löe.** 1964. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol. Scan. **22**:121–135.

31. **Slots, J.** 1977. The predominant cultivable microflora of advanced periodontitis. Scand. J. Dent. Res. **85**:114–121.
32. **Sundqvist, G. K., M. I. Eckerbom, Å. P. Larsson, and U. T. Sjörgren.** 1979. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. Infect. Immun. **25**:685–693.
33. **Sutter, V. L., and S. M. Finegold.** 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. Antimicrob. Agents Chemother. **10**:736–752.
34. **Sutter, V. L., M. J. Jones, and A. T. M. Ghoneim.** 1983. Antimicrobial susceptibilities of bacteria associated with periodontal disease. Antimicrob. Agents Chemother. **23**: 483–486.
35. **Syed, S. A., and W. J. Loesche.** 1972. Survival of human dental plaque flora in various transport media. Appl. Microbiol. **24**:638–644.
36. **Syed, S. A., M. Svanberg, and G. Svanberg.** 1980. The predominant cultivable dental plaque flora of beagle dogs with gingivitis. J. Periodontal Res. **15**:123–136.
37. **Tanner, A. C. R., S. Badger, C.-H. Lai, M. A. Listgarten, R. A. Visconti, and S. S. Socransky.** 1981. *Wolinella* gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin et al.) comb. nov., and description of *Bacteroides gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from humans with periodontal disease. Int. J. Syst. Bacteriol. **31**:432–445.
38. **Tanner, A. C. R., C. Haffer, G. T. Bratthall, R. A. Visconti, and S. S. Socransky.** 1979. A study of the bacteria associated with advancing periodontitis in man. J. Clin. Periodontol. **6**:278–307.
39. **Thornsberry, C., and L. K. McDougal.** 1982. Ampicillin resistant *Haemophilus influenzae*. I. Incidence, mechanism and detection. Postgrad. Med. **71**:133–145.
40. **Valdés, M. V., P. M. Lobbins, and J. Slots.** 1982. Beta-lactamase producing bacteria in the human oral cavity. J. Oral Pathol. **11**:58–63.
41. **von Konow, L., and C. E. Nord.** 1983. Ornidazole compared to phenoxymethylpenicillin in the treatment of orofacial infections. J. Antimicrob. Chemother. **11**:207–215.
42. **von Konow, L., C. E. Nord, and A. Nordenram.** 1981. Anaerobic bacteria in dentoalveolar infections. Int. J. Oral Surg. **10**:313–322.
43. **Walker, C. B., T. A. Niebloom, J. M. Gordon, and S. S. Socransky.** 1980. In vitro susceptibilities of bacterial from human periodontal pockets to 13 antimicrobial agents, p. 508–511. *In* J. D. Nelson and C. Grassi (ed.), Current Chemotherapy and Infectious Disease, vol. 1. American Society for Microbiology, Washington, D.C.
44. **Whitcher, B. L., O. R. Bierne, and R. A. Smith.** 1983. Beta-lactamase-producing *Bacteroides melaninogenicus* and osteomyelitis of the mandible. J. Oral Med. **38**:17–20.
45. **White, D., and D. Mayrand.** 1981. Association of oral *Bacteroides* with gingivitis and adult periodontitis. J. Periodontal Res. **16**:259–265.
46. **Williams, B. L., G. F. McCann, and F. D. Schoenknecht.** 1983. Bacteriology of dental abscesses of endodontic origin. J. Clin. Microbiol. **18**:770–774.