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## Antibiotic Susceptibility of Black-Pigmented *Bacteroides* Isolates from the Human Oral Cavity

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The minimal inhibitory concentrations of penicillin and six other antibiotics were determined for 66 oral black-pigmented *Bacteroides* isolates by using the National Committee for Clinical Laboratory Standards proposed standard agar dilution technique. These results plus iodometric determination of  $\beta$ -lactamase activity showed that oral isolates of black-pigmented *Bacteroides* are remaining relatively susceptible to commonly used antibiotics.

The black-pigmented *Bacteroides* species (BPB) (*B. melaninogenicus* and *B. asaccharolyticus*) are commonly found as normal flora in the human intestinal and reproductive tracts and in the oral cavity (3). Their incidence within the oral cavity seems to increase with the eruption of permanent teeth (13), and, along with other normal oral flora, they are characteristically involved in endodontic as well as periodontic infections (4, 13).

The significance of *B. melaninogenicus* in endodontic infections was first shown in 1976 by Sundqvist, who studied the microbial flora of teeth with pulpal necrosis (G. Sundqvist, Umea University Odontological Dissertation, Umea, Sweden, 1976). He found that only those teeth with periapical destruction contained bacteria and that symptoms of acute periapical inflammation were present only in teeth containing *B. melaninogenicus* as part of the microbial flora. Griffie et al. in 1980 demonstrated a similar relationship between periapical symptoms and the presence of *B. melaninogenicus* (4).

Penicillin has traditionally been the recommended chemotherapy for most infections involving BPB. However, a number of recent studies have suggested an increasing resistance of these organisms to penicillin and to other commonly prescribed antibiotics (1, 2, 5, 6, 8, 10). Although this has aroused concern among dental clinicians, it cannot be determined from these studies whether BPB present as part of the normal oral microflora show similar patterns of increasing resistance. These studies were conducted in hospital laboratories and evaluated antibiotic susceptibilities of BPB isolates originating from a variety of unspecified body sites and clinical specimens. Only the study of Niederau et al. (8) included oral isolates, but the number of isolates tested was small ( $n = 10$ ).

The purpose of our study was to determine the antibiotic susceptibility of oral isolates of BPB from healthy mouths. These data will aid in establishing a protocol for therapy since these isolates are constituents of the normal oral flora and it is this normal flora which serves as the source of pulpal, periapical, and periodontal infections.

A total of 66 BPB isolates were obtained from specimens of subgingival plaque collected from freshman dental students ( $n = 9$ ) and patients seen in the dental hygiene clinic ( $n = 53$ ) at the Marquette University School of Dentistry, Milwaukee, Wis., as well as from pulps of patients undergoing endodontic therapy in private practice ( $n = 4$ ). Since the results did not reveal any significant differences in the antibiotic susceptibility of these isolates, no distinction between the groups is made below.

Plaque specimens were collected into reduced thioglycollate medium without indicator (Difco Laboratories, Detroit, Mich.) and dispersed by shaking with a glass bead. Each broth was inoculated onto a brain heart infusion agar plate (Difco) supplemented with 0.5% yeast extract (Difco), 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.), 0.5  $\mu$ g of vitamin K (Sigma Chemical Co., St. Louis, Mo.) per ml, 0.5  $\mu$ g of hemin (Sigma) per ml, and 5% laked sheep blood (GIBCO Diagnostics, Madison, Wis.). The plates were incubated anaerobically at 35 to 37°C for 5 to 7 days with the GasPak System (BBL). Colonies that produced brown to black pigmentation were subcultured to two supplemented brain heart infusion agar plates. One was incubated anaerobically, and the other was incubated aerobically in an atmosphere of 5 to 10% CO<sub>2</sub>. Isolates were identified as members of the *B. melaninogenicus*-*B. asaccharolyticus* group if they produced a brown to black pigment

on the anaerobic plate, were incapable of growth on the aerobic plate, appeared as gram-negative bacilli or coccobacilli upon staining, and showed a positive immunofluorescent reaction with the Fluoretec-M kit (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.). Previous studies have shown that the Fluoretec-M kit is a reliable method for identifying *B. melaninogenicus* and *B. asaccharolyticus* (12).

The following antibiotics were used in testing: erythromycin, cephalixin, and potassium penicillin G (Eli Lilly & Co., Indianapolis, Ind.); clindamycin hydrochloride (The Upjohn Co., Kalamazoo, Mich.); tetracycline hydrochloride and chloramphenicol (Sigma); and ampicillin (Bristol Laboratories, Syracuse, N.Y.). Appropriate stock solutions of each antibiotic were prepared in sterile water and stored at  $-20^{\circ}\text{C}$  for not more than 1 week before use.

Antibiotic susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards proposed standard agar dilution procedure (7). Anaerobic and aerobic growth control plates without antibiotic were included in each experiment. Due to the fastidious nature of the BPB, we found it necessary to add rabbit serum (GIBCO Laboratories, Grand Island, N.Y.) to a 5% concentration to the thioglycollate medium and to Wilkins Chalgren agar (Difco). Preliminary experiments showed that this enhanced the growth and recovery of BPB without changing the acceptable range of minimal inhibitory concentration (MIC) values required for the control cultures.

The Wilkins Chalgren agar plates were incubated anaerobically at 35 to  $37^{\circ}\text{C}$  for 48 h. The MIC for each isolate was defined as the lowest concentration of antibiotic yielding no growth or a barely visible haze of growth.

The  $\beta$ -lactamase test was performed on 41 isolates according to the rapid iodometric method (11). Known positive and negative control strains were assayed simultaneously. This method was chosen instead of the chromogenic cephalosporin method since  $\beta$ -lactamases of BPB are more active against penicillin than against cephalothin (9).

The results of the agar dilution susceptibility tests are shown in Table 1. The MIC values of chloramphenicol, clindamycin, penicillin G, and tetracycline for control cultures of *Clostridium perfringens* (ATCC 13124) and *Bacteroides thetaiotaomicron* (ATCC 29741) fell within the acceptable range (mode MIC  $\pm$  one dilution). MIC values of the other antibiotics tested have not been established for the control strains in the proposed standard.

We found that 90% of our BPB isolates were inhibited by penicillin G at 0.5 U/ml or ampicillin at 0.5  $\mu\text{g}/\text{ml}$ . The six isolates which had penicil-

TABLE 1. Inhibitory activity of antibiotics against oral isolates of BPB

Antibiotic	MIC ( $\mu\text{g}/\text{ml}$ ) <sup>a</sup>		
	Range <sup>b</sup>	For 50% inhibition	For 90% inhibition
Penicillin G	$\leq 0.125$ –8	$\leq 0.125$	0.5
Ampicillin	$\leq 0.125$ –4	$\leq 0.125$	0.5
Tetracycline	$\leq 0.125$ –16	$\leq 0.125$	4
Cephalexin	$\leq 0.125$ –16	0.25	0.5
Erythromycin	$\leq 0.125$ –1	$\leq 0.125$	0.25
Clindamycin	$\leq 0.125$	$\leq 0.125$	$\leq 0.125$
Chloramphenicol	0.5–2	1	1

<sup>a</sup> Penicillin G MICs are expressed as units per milliliter.

<sup>b</sup> Number of isolates ( $n$ ) = 66 for all antibiotics except clindamycin, for which  $n$  = 52.

lin MIC values of  $>0.5$  U/ml also had ampicillin MIC values of  $>0.5$   $\mu\text{g}/\text{ml}$ . In contrast, recent studies of isolates of unspecified, presumably nonoral, origin have found that penicillin and ampicillin levels from 4 to  $>64$  U or  $\mu\text{g}/\text{ml}$  were required for 90% inhibition (1, 2, 5, 6, 10; K. E. Aldridge, C. V. Sanders, and R. L. Marier, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C21, p. 266). Only the very recent study by Sutter et al. (V. L. Sutter, M. J. Jones, and A. T. Ghoneim, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, A18, p. 4) reports data similar to ours. Using isolates from periodontitis patients, Sutter et al. found that 90% of their BPB isolates were inhibited by penicillin G at 0.5 U/ml, with a few strains requiring  $>32$  U/ml for inhibition.

A total of 41 of our isolates were tested for  $\beta$ -lactamase production. These included 37 isolates with penicillin MIC values of  $\leq 0.5$  U/ml and 4 isolates with penicillin MIC values of 4 to 8 U/ml. All isolates with penicillin MICs of  $\leq 0.5$  U/ml were negative for  $\beta$ -lactamase, whereas those with penicillin MICs of 4 to 8 U/ml were positive. In contrast to Murray and Rosenblatt, who found 56% of their clinical isolates to be  $\beta$ -lactamase producing (6), only 10% of our oral isolates tested exhibited this activity. The clinical significance of this enzyme in anaerobes, however, is still questionable.

Ninety percent of our isolates were inhibited by tetracycline at 4  $\mu\text{g}/\text{ml}$ . Studies from 1977 and 1980 reported only 40 to 50% inhibition of BPB at this concentration (2, 6). We also found that cephalixin at 0.5  $\mu\text{g}/\text{ml}$  was sufficient to produce 90% inhibition. This contrasts greatly with the report of Niederau et al., who found that cephalixin at 25  $\mu\text{g}/\text{ml}$  was necessary for 90% inhibition (8). Additionally, only Niederau et al. have demonstrated increased resistance to chloramphenicol, finding that 25  $\mu\text{g}/\text{ml}$  was needed for 90% inhibition. No other studies, including ours,

have reported increasing resistance to this antibiotic. Likewise, no studies have shown the BPB to be developing resistance to clindamycin or erythromycin, regardless of the source or nature of the specimen.

Our data indicate that oral isolates of BPB are remaining relatively susceptible to penicillin and other antibiotics. Other authors have suggested that their increased isolation of resistant strains may have been due to extensive antibiotic use at their hospitals (1, 2). This may account for our isolation of highly susceptible BPB, since our isolates were derived from patients who were not recently hospitalized. Even patients with endodontic and periodontic infections would be treated as outpatients. Our patient population was derived from a broad range of age and socioeconomic groups; therefore we feel that resistant strains were not missed as a result of evaluating a limited or perhaps biased sample.

The increasing isolation rate of antibiotic-resistant strains from clinical specimens may or may not foretell an increasing incidence among the normal oral microflora. Our results suggest that data generated in clinical laboratories may not be applicable to endodontic and periodontic patients or outpatient groups. Periodic monitoring of the susceptibility patterns of oral microorganisms may be appropriate and yield useful data for the dental clinician and oral surgeon in the form of a more up-to-date protocol for therapy.

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