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Penicillin Resistance and Penicillinase Production in Clinical Isolates of *Bacteroides melaninogenicus*

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The minimum inhibitory concentrations (MIC) of penicillin and six other antimicrobials were determined for 50 clinical isolates of *Bacteroides melaninogenicus*. Agar dilution susceptibilities were performed using supplemented brucella blood agar and the proposed National Committee for Clinical Laboratory Standards standard method for anaerobes; results with the two methods were comparable. A penicillin concentration ≥0.8 μg/ml was needed to inhibit 56% of the isolates, whereas 100% were susceptible to 0.1 μg of clindamycin per ml. All isolates with penicillin MIC values ≥0.8 μg/ml produced β-lactamase using a slide method. A micro-iodometric assay was used to quantitate β-lactamase production in six isolates. The β-lactamase activity of *B. melaninogenicus* was comparable to that of a *Staphylococcus aureus* isolate but was not inducible, and the specific amount produced correlated only partially with penicillin MIC values. A clinical review of patients from whom the β-lactamase-producing strains of *B. melaninogenicus* were isolated did not suggest any increased virulence in these strains or an unexpectedly poor clinical response to appropriate therapy.

The susceptibility of anaerobes to antimicrobial agents is, in most instances, predictable. Generally, penicillin G is effective against anaerobes, with the exception of *Bacteroides fragilis* and some strains of *Fusobacterium varium* and *Clostridium* species (2, 3). The mechanism of *B. fragilis* resistance to penicillin is unknown, although it may be related to the production of β-lactamase. This enzyme is more active against cephalosporins than penicillins, is present in very low concentrations, and is associated with the bacterial cell membranes and released only with the disruption of the cell (1, 6, 7). β-Lactamase production by other anaerobes had not been detected until A. S. Hackman and J. Wong (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A9, p. 2) recently reported the isolation of β-lactamase-producing strains of *Bacteroides melaninogenicus*. Although *B. melaninogenicus* is commonly considered to be susceptible to penicillin, previous studies from our laboratory demonstrated that a concentration of penicillin of 12.5 μg/ml or greater was required for the inhibition of 17% of the clinical isolates of *B. melaninogenicus* (5, 8). These data and the report of Hackman and Wong (above) prompted us to investigate further the antimicrobial susceptibilities of *B. melaninogenicus* and to determine the frequency and significance of β-lactamase production in our isolates.

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MATERIALS AND METHODS

B. melaninogenicus isolates were recovered from clinical specimens submitted to our clinical microbiology laboratory between October 1975 and May 1976. Isolation and identification of the isolates were performed according to the methods detailed in the Virginia Polytechnic Institute (4) and the Wadsworth (9) anaerobe laboratory manuals. Subcultures of the isolates were made at the time of identification, and equal volumes of skim milk (Baltimore Biological Laboratory, Cockeysville, Md.) and the culture in thioglycolate broth (enriched with hemin, 5 μg/ml, and vitamin K, 0.1 μ/ml) were mixed and stored at −60°C.

Antimicrobial susceptibility testing was performed in an anaerobic chamber by the agar dilution method as described by Sutter et al. (9). The antimicrobial agents in appropriate dilutions in sterile distilled water were incorporated into a brucella agar base enriched with 5% whole defibrinated sheep blood and vitamin K, 0.1 μg/ml. The concentration of metronidazole was calculated on a dry-weight basis; the remaining antimicrobials were diluted on the basis of the specific activity. Susceptibility tests were also performed using the proposed National Committee for Clinical Laboratory Standards standard method for anaerobes. The results were comparable for the two methods.

β-Lactamase production by isolates of *B. melaninogenicus* was determined by two methods, the

slide modification of the iodometric method (J. E. Rosenblatt and A. M. Neumann, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 388, 1975) and the quantitative micro-iodometric assay described by Sykes and Nordström (10). For the slide iodometric assay, a heavy loopful of colonies was removed from a blood agar plate and emulsified on a slide in a drop of phosphate-buffered saline (pH 6.4) containing iodine (3 mg/ml), potassium iodide (15 mg/ml), and aqueous pencillin G (50 mg/ml). One drop of 0.4% starch in water was then added, and the mixture was observed for 5 min. Although the mixture may initially be purple-colored, a positive reaction will be white or colorless within 5 min. The inoculum for the micro-iodometric assay of β-lactamase was prepared with isolates of *B. melaninogenicus* that were grown for 48 h in brucella broth supplemented with hemin and vitamin K. Each broth was then divided into two aliquots; penicillin G (100 μg/ml) was added to one aliquot in an attempt to induce β-lactamase production and the broths were reincubated for 4 h. The broths were then centrifuged, and the bacterial pellets were washed twice in phosphate-buffered saline at pH 5.9 and frozen at −60°C. Before the determination of enzyme activity, the bacteria were frozen and thawed three times and adjusted to an absorbance of 0.25 at 620 nm. The quantitative assay of β-lactamase activity was expressed in units defined as the amount of enzyme that hydrolyzed benzylpenicillin at the rate of 1 μmol/min at 30°C and pH 5.9.

RESULTS

Fifty isolates representing all three subspecies of *B. melaninogenicus* were recovered from various clincal sources. The minimum inhibitory concentrations (MICs) of seven antibiotics against 46 or 50 of these isolates are presented in Table 1. A penicillin concentration of 0.8 μg/ml was needed to inhibit 48% of the isolates. Fifty micrograms of carbenicillin per milliliter inhibited 100% of the isolates. At 3.1 μg/ml, cephalothin inhibited 46% of the isolates, and all isolates tested were susceptible to 0.1 μg of clindamycin per ml. Only 43% of the isolates were inhibited by 3.1 μg of tetracycline per ml, whereas 6.2 μg of chloramphenicol per ml inhibited all the isolates. All but 1 of the 46 isolates were inhibited by 3.1 μg of metronidazole per ml. In the isolates reported herein, resistance to β-lactam antibiotics was not associated with resistance to other antibiotics.

TABLE 1. *Inhibitory activity of antibiotics against isolates of B. melaninogenicus*

Antibiotic	No. of isolates tested	Cumulative % inhibited at concn (μg/ml) of:			
		0.8	3.1	6.2	50
Penicillin	50	48	62	74	98
Carbenicillin	46	43	72	85	100
Cephalothin	46	41	46	46	93
Clindamycin	50	100			
Tetracycline	46	28	43	54	93
Chloramphenicol	46	26	85	100	
Metronidazole	46	67	98	98	100

All isolates with penicillin MIC values ≥0.8 μg/ml and cephalothin MIC values ≥1.6 μg/ml produced detectable β-lactamase using the slide method (Table 2). Twenty-seven of the 28 isolates with carbenicillin MIC values ≥0.8 μg/ml produced β-lactamase. There was no difference in the qualitative production of β-lactamase among the three *B. melaninogenicus* subspecies.

β-Lactamase production was quantitated by the micro-iodometric assay for six isolates of *B. melaninogenicus* that had penicillin MIC values ranging from 0.1 to 50 μg/ml (Table 3). The β-lactamase activity of *B. melaninogenicus* was equal to or greater than that of the *Staphylococcus aureus* isolate not induced by pre-exposure to penicillin. In addition, β-lactamase production by isolates of *B. melaninogenicus* was not inducible with penicillin, whereas β-lactamase production by *S. aureus* was inducible. The specific amount of β-lactamase produced correlated only partially with the MIC values of penicillin.

DISCUSSION

Previous studies from our laboratory (5, 8) have characterized the in vitro antimicrobial susceptibilities of anaerobes isolated from clinical material and have provided data with which to compare the results reported in the present study. The isolates of *B. melaninogenicus* examined in the present study and in the earlier

TABLE 2. *Correlation of penicillin and cephalothin MICs and β-lactamase activity (slide test) in isolates of B. melaninogenicus*

MIC (μg/ml)	Penicillin[a]		Cephalothin[b]	
	No. tested	No. with β-lactamase activity	No. tested	No. with β-lactamase activity
0.1	21	0	9	0
0.2	1	0	5	0
0.4			4	0
0.8	2	2	1	0
1.6	1	1	1	1
3.1	6	6	1	1
6.2	6	6		
12.5	7	7	7	7
25	3	3	8	8
50	2	2	7	7
100	1	1	2	2
>100			1	1

[a] Fifty-six percent with β-lactamase activity.
[b] Fifty-nine percent with β-lactamase activity.

TABLE 3. *Quantitation of β-lactamase activity (micro-iodometric assay) in isolates of B. melaninogenicus*

Isolate	Penicillin MIC (μg/ml)	β-Lactamase activity (IU × 10^{-3})	
		Without penicillin pre-exposure	With penicillin pre-exposure
S. aureus	1.0	0.59	1.72
B. melaninogenicus subsp.			
intermedius	0.1	ND[a]	ND
melaninogenicus	1.6	0.78	0.81
asaccharolyticus	6.2	1.19	1.16
asaccharolyticus	25	0.95	0.99
intermedius	50	1.43	1.40
melaninogenicus	50	0.61	0.59

[a] ND, Not detectable.

studies were highly susceptible to clindamycin, chloramphenicol, and, with one exception reported herein, metronidazole. An increase in resistance to tetracycline was found. Only 43% of the isolates in this study were inhibited at 3.1 μg/ml or less, compared with 79% of the isolates of *B. melaninogenicus* reported in 1972. The β-lactam antibiotics were not as effective in the current study, compared with the earlier results. Although the production of β-lactamase was not examined in previous studies, 38 and 50% of the isolates of *B. melaninogenicus* reported in 1972 and 1974, respectively, had penicillin MIC values ≥0.8 μg/ml, the concentration at which β-lactamase production was detected in the present study. In contrast, 28 of 50 (56%) of the current isolates had penicillin MIC values ≥0.8 μg/ml. Less than 0.8 μg of carbenicillin per ml inhibited 50 and 39% of the isolates reported in 1974 and herein, respectively. Less than 1.6 μg of cephalothin per ml was effective against 69% of the isolates reported in 1972 and 41% of those studied herein. Although the decreased effectiveness of the β-lactam antibiotics could be the result of technical differences in antimicrobial susceptibility testing, it is significant that more than half of the isolates of *B. melaninogenicus* examined in this study produced β-lactamase with activity against both penicillins and cephalosporins.

β-Lactamase production was initially detected with the slide modification of the starch agar iodometric method and was observed in all three subspecies of *B. melaninogenicus*. In contrast to the report of Hackman and Wong (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A9, p. 2), the amount of enzyme produced was similar for the three subspecies and was substantially greater than we have detected in isolates of *B. fragilis*, although similar to that detected in *S. aureus*. As has been noted with *B. fragilis*, the enzyme activity was not inducible with penicillin and was only roughly correlated with the MIC values.

To determine the effect of β-lactamase production by *B. melaninogenicus* on the management of anaerobic infections, the clinical histories of 28 patients from whom β-lactamase-producing isolates had been recovered were reviewed. In 14 of the 28 patients, we were unable to determine the clinical significance of the isolate; and in the other 14 patients, the isolate was from a source where infection was either obvious or likely. Of the latter 14 patients, 12 were appropriately treated with an effective antimicrobial agent or surgical drainage and debridement (or both) when indicated. Of these 12 patients, 6 received clindamycin and 4 received therapeutic doses of a penicillin plus surgery. Two other patients were surgically treated but did not receive adequate antibiotic therapy. One patient with an isolate from two separate blood cultures had a self-limiting bacteremia, and another with endometritis was treated only with cephalothin (MIC = 50 μg/ml). Thus, this brief clinical review did not suggest any increased virulence for β-lactamase-producing isolates of *B. melaninogenicus* nor any evidence of poor clinical response to appropriate treatment, including penicillin therapy.

This study shows that most (56%) of the clinical isolates of *B. melaninogenicus* produce β-lactamase. The clinical significance of this finding is in doubt because the enzyme does not appear to be inducible, and our brief clinical review did not suggest a poor clinical outcome in patients yielding these isolates. However, because approximately 25 and 40% of our isolates had penicillin MIC values ≥12.5 and 6.2 μg/ml, respectively, knowledge of specific susceptibility results seems to be important. When these data are not routinely available, the simple slide iodometric test can detect β-lactamase-producing isolates of *B. melaninogenicus*.

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