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Bacteroides Penicillinase

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The resistance of most strains of *Bacteroides* to penicillin stimulated this study of penicillinase production. Garrod (2) suspected penicillinase production in two strains of *B. fragilis*, but Holt and Stewart (3) found neither β-lactamase nor amidase activity in eight strains of *Bacteroides* (species unspecified).

Inactivation of benzylpenicillin, ampicillin, and oxacillin by penicillinase was determined by the disc method (Fig. 1) and antibiotic sensitivity by serial dilution in agar. Among 31 *Bacteroides* strains from our patients and 9 from other laboratories, we found penicillinase produced by 2 of 11 that were inhibited by 5 μg of benzylpenicillin per ml and by 14 of 29 more resistant strains. Ampicillin was inactivated by 1 of 8 strains inhibited by 1.25 μg of ampicillin per ml and 10 of 32 more resistant strains. Among 28 tested, only 2 strains of *B. funduliformis* and one *Bacteroides* species inactivated oxacillin.

Reaction mixtures of 12 strains that inactivated benzylpenicillin and ampicillin and 3 strains that inactivated oxacillin were examined by a thin-layer chromatographic method, adapted for this study from Alicino's iodometric assay (1).

A 2-ml amount of each of the following was added to 3 ml of 0.05 M phosphate buffer, *p*H 7.0, containing 600 to 750, μg of benzylpenicillin or ampicillin per ml or 120 to 160 μg of oxacillin per ml: a 48- to 72-hr thioglycollate broth culture; its membrane filtrate (obtained by filtration through an 0.5-μm filter, Millipore Corp., Bedford, Mass.); the supernatant fluid from centrifuged cells; or control sterile thioglycollate broth. Reaction mixtures were incubated aerobically at 37 C for 18 hr. Portions (1 to 2 μliters) were analyzed by thin-layer chromatography on precoated cellulose plates (80 μm thick; Brinkman Instruments, Inc., Westbury, N.Y.). Aerobic penicillinase producers were ruled out by cultures at each step. Chromatograms were developed in 79% ethyl alcohol or 83% methanol (for ampicillin) for 2 hr, dried, sprayed with 0.5 N NaOH, and dried again; they were then sprayed with a mixture containing glacial acetic acid, 4% KI in 0.1 N iodine, and 1% soluble starch (1:3:50, v/v). The antibiotics and hydrolysis products rapidly decolorized the iodine reagent.

Hydrolysis of benzylpenicillin invariably gave a product with the R_F of benzylpenicilloic acid. The same results were obtained with 13 strains tested at *p*H 8.5 for acylase activity, and no products were found with the R_F of 6-amino penicillanic acid or the penicilloic acid of 6-amino penicillanic acid. Benzylpenicillin, benzylpenicilloic acid, and 6-amino penicillanic acid were well separated in the solvent system described. Hydrolysis of ampicillin by *Bacteroides* penicillinase also gave a product with the same R_F as its penicilloic acid. [Ampicillin is readily separated from the penicilloic acid of ampicillin and 6-amino penicillanic acid, but the penicilloic acid acid of ampicillin does not separate well from

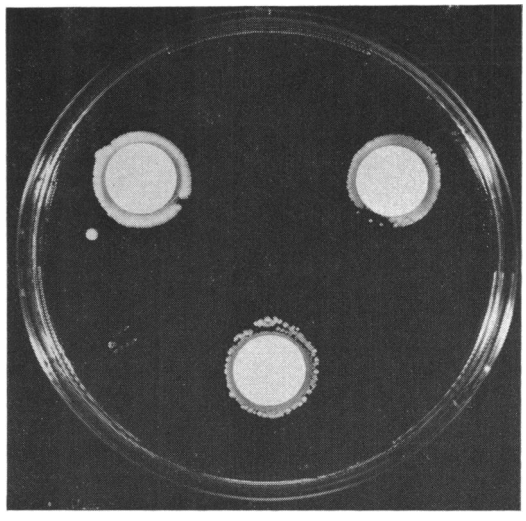

FIG. 1. *Paper discs (no. 57 GH, Schleicher and Schuell Co., Keene, N.H.) contained 0.8 ml of membrane filtrates (Millipore Corp.) of thioglycollate cultures of three different strains of B. fragilis. They were placed on agar containing 0.05 μg of benzylpenicillin per ml and streaked with Sarcina lutea. Penicillinase produced yellow growth of penicillin-sensitive S. lutea around the disc after incubation at 37 C. Penicillinase was also demonstrated with discs dipped into 48- to 72-hr turbid thioglycollate cultures of Bacteroides. Growth of Bacteroides in the discs was prevented by the aerobic incubation of the S. lutea plates.*

TABLE 1. *Relationship of penicillinase activity to antibiotic resistance*[a]

Species of Bacteroides	Penicillin derivatives	Minimal inhibitory concn (μg/ml)						Total[b]
		0.312	1.25	5	10	20	NI	
B. fragilis	BP			0/1	2/8	1/7	2[c]/2	5/18
	AM		0/3	1[c]/11	0/3		1[c]/1	2/18
B. funduliformis	BP			0/1	2[c]/5	3[c]/3	1[c]/1	6/10
	AM		0/1	2[c]/6	3[c]/3			5/10
B. melaninogenicum	BP			0/3				0/3
	AM			0/2	0/1			0/3
B. oralis	BP		1[c]/5					1/5
	AM	0/1	1/2	1[c]/2				2/5
Unidentified	BP		1[c]/1		1[c]/1		1[c]/1	3/3
	AM				1/2[c]		1[c]/1	2/3

[a] Numerator, strains hydrolyzing benzylpenicillin (BP) or ampicillin (AM); denominator, strains inhibited at that concentration of benzylpenicillin or ampicillin; NI, not inhibited.

[b] Penicillinase was produced by 7 of 39 inhibited by 5 μg of either antibiotic and 19 of 39 resistant to 5 μg ($P = <.01$).

[c] β-Lactamase demonstrated chromatographically.

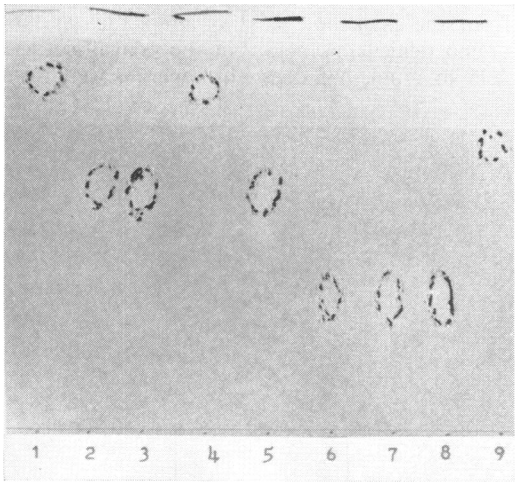

FIG. 2. *Thin-layer chromatography of reaction mixtures containing membrane filtrates (Millipore Corp.) of two strains of Bacteroides and benzylpenicillin or ampicillin developed with 83% methanol; 1, 4 = benzylpenicillin, 0.6 μg; 2, 3 = hydrolysis products of two strains of Bacteroides in benzylpenicillin; 5 = benzylpenicilloic acid, 0.5 μg; 6 = penicilloic acid of ampicillin, 0.5 μg; 7, 8 = hydrolysis products of two strains of Bacteroides in ampicillin and 9 = ampicillin, 0.6 μg.*

6-amino penicillanic acid. We performed a second chromatography with a solvent system containing methanol, water, and acetic acid (25:10:5, v/v) to confirm the identity of the hydrolysis product of ampicillin.] This β-lactamase activity in *Bacteroides* is illustrated in Fig. 2 and was also observed with three strains when oxacillin was used as a substrate. These strains converted oxacillin to compounds with the same R_F as those produced from oxacillin by alkaline hydrolysis of excess β-lactamase (*Bacillus cereus*). The strongest and most consistent activity developed after centrifugation or by filtration of *Bacteroides*, perhaps, because disruption of bacteria released the enzyme (Table 1). The β-lactamase activity against three penicillin derivatives does not necessarily represent three different classes of this enzyme since it could be produced by one enzyme with apparent rate constants for hydrolysis in the order: benzylpenicillin > ampicillin > oxacillin.

More frequent penicillinase activity among more resistant strains suggests that resistance of *Bacteroides* to penicillin may sometimes result from this enzyme.

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