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Role of *Bacteroides bivius* β-Lactamase in β-Lactam Susceptibility

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The susceptibility of 46 clinical isolates of *Bacteroides bivius* to amoxicillin, cefotaxime, cefoxitin, ceftizoxime, cephaloridine, cephalothin, moxalactam, penicillin G, amoxicillin plus clavulanic acid in a ratio of 2:1, carbenicillin, cefamandole, and ceftazidime was determined by an agar dilution technique. For the first eight agents susceptibility testing was also done with the addition of clavulanic acid (0.75 μg/ml). For all agents, β-lactamase-positive strains (35, using a nitrocefin slide test) were inhibited at higher concentrations than β-lactamase-negative strains. Clavulanic acid reduced the susceptibility of the β-lactamase-positive strains to the level of the β-lactamase-negative strains to all agents. We prepared crude extracts of β-lactamase from six strains. Activity against nitrocefin was directly related to their susceptibilities. The β-lactamase had a mixed-substrate profile, hydrolyzing both penicillins and cephalosporins. Our results suggest a slow inactivation of cefoxitin, ceftizoxime, and moxalactam by the β-lactamase. Clavulanic acid and cefoxitin inhibited the enzyme, whereas *p*-hydroxymercuribenzoate and cloxacillin did not. Thus, there was a clear relationship between β-lactamase activity and susceptibility to β-lactams, including cefoxitin and third-generation cephalosporins. The substrate and inhibition profiles of the *B. bivius* β-lactamase were different from those of enzymes found in the "*B. fragilis* group."

Bacteroides bivius is an obligately anaerobic, nonspore-forming, nonmotile, gram-negative rod, formerly known as the "PS group" (9), which often produces a detectable β-lactamase (3, 13, 21). This microorganism is part of the normal vaginal flora (4, 12, 20, 23) and has been more frequently isolated than *B. fragilis* in gynecological-obstetric infections (1, 4–6, 11, 21).

The purpose of this work was to study the role of the β-lactamase in the susceptibility of *B. bivius* to various β-lactams and to determine some of the characteristics of the enzyme.

We looked for the presence of β-lactamase in 46 clinical isolates of *B. bivius*. We determined the in vitro susceptibilities of these strains to various β-lactams, including cefotaxime, cefoxitin, ceftizoxime, and moxalactam, with and without the addition of clavulanic acid. In some isolates we extracted the β-lactamase, measured its activity, and determined its substrate and inhibition profiles.

MATERIALS AND METHODS

Bacterial strains. Forty-six strains of *B. bivius* isolated from clinical specimens in the microbiology laboratory of Hôpital St-Luc (Montreal, Canada) were studied. Each isolate was obtained from a different patient. The strains were identified by the methods outlined in the fourth edition of the *Anaerobe Laboratory Manual* of the Virginia Polytechnic Institute (8) with commercially available media (Carr-Scarborough Microbiological Inc., Ann Arbor, Mich.). *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124 were obtained from the American Type Culture Collection (Rockville, Md.).

Detection of β-lactamase. β-Lactamase activity was detected by using a slide test technique with nitrocefin (Glaxo Ltd., Greenford, England) (2).

Susceptibility testing. MICs were determined by the agar dilution method proposed by the National Committee for Clinical Laboratory Standards (15), using Wilkins-Chalgren agar (29). Overnight cultures of the organisms in thioglycolate broth without indicator (BBL Microbiology Systems, Mississauga, Canada) supplemented with hemin (5 μg/ml) and menadione (1 μg/ml) and adjusted to equal one-half the density of a McFarland no. 1 standard were used as inocula. A Steers replicator (22) delivering 1 μl was used to inoculate the plates, which were incubated at 37°C for 48 h in an anaerobic jar (GasPak; BBL). The MIC was read as the lowest concentration of antibiotic allowing no growth or a faint indistinct haze. The β-lactams were kindly provided by the following suppliers: amoxicillin (Beecham Laboratories, Pointe-Claire, Canada); carbenicillin and penicillin G (Ayerst Laboratories, Montreal, Canada); cefamandole, cephalothin, and moxalactam (Eli Lilly & Co. Canada, Scarborough, Canada); cefotaxime (Roussel Canada Inc., Montreal, Canada); cefoxitin (C. E. Frosst and Co., Pointe-Claire, Canada); ceftazidime and cephaloridine (Glaxo Ltd., Toronto, Canada); ceftizoxime (Smith Kline & French Canada Ltd., Mississauga, Canada). For amoxicillin, cefotaxime, cefoxitin, ceftizoxime, cephaloridine, cephalothin, moxalactam, and penicillin G the MICs were also determined with the addition of 0.75 μg of clavulanic acid (Beecham Laboratories) per ml. Each dilution was poured simultaneously in two series of plates, one of which contained the clavulanic acid. MICs were also determined for the combination of amoxicillin and clavulanic acid in a ratio of 2:1 (wt/wt).

β-Lactamase extraction. The organisms were cultured in 500 ml of the following medium: Difco Proteose Peptone, 20 g, and yeast extract, 5 g (Difco Laboratories Inc., Detroit Mich.); sodium chloride, 5 g, calcium carbonate, 2 mg, and cysteine hydrochloride, 0.55 g (Fisher Scientific Co. Ltd., Montreal, Canada); hemin, 5 mg, and resazurin, 1 mg (Eastman Kodak Co., Rochester, N.Y.); sodium bicarbonate, 1 g (Matheson Coleman & Bell, Norwood, Ohio); glucose, 10 g (BBL); and menadione, 1 mg (ICN Canada Ltd., Montreal, Canada). This is a modification of the medium used by Olsson et al. (19) for the extraction of β-lactamase from *Bacteroides* species. The inoculated medium was incubated at 37°C in an anaerobic jar (GasPak jar). At

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mid-logarithmic phase (0.6 to 0.8 absorbance at 660 nm) the cells were harvested and washed twice in 0.05 M phosphate buffer, pH 7.0, containing 1.0 mM dithiothreitol (Sigma Chemical Co., St. Louis, Mo.). The cells were suspended in a final volume of 10 to 15 ml in the same buffer. Two milliliters was taken for dry weight measurement and the remaining bacteria were disrupted with a Sonifier cell disruptor model 350 (Branson Sonic Power Co., Danbury, Conn.) with a 0.125-in. (ca. 0.318-cm) microtip. The cells were sonicated by using 1-min pulses followed by 1 min of cooling until most of the cells were broken. For isoelectric focusing the cells were broken with a French press (American Instrument Co., Silver Spring, Md.). The cells were passed five times in the press to be sure that most of the cells were disrupted. The broken cells were centrifuged at 30,000 × *g* for 20 min (22,000 rpm in a 40.2 rotor [Beckman Instruments, Inc., Palo Alto, Calif.]). The supernatant was used as the crude β-lactamase extract.

β-Lactamase assays. Cephalosporinase activity of the crude extracts was measured by the spectrophotometric method of O'Callaghan et al. (17). The wavelengths used for measuring change in absorption maxima of β-lactam rings were 262 nm for cephalothin (7), 274 nm for cefamandole (7), 264 nm for cefotaxime (7), 255 nm for cephaloridine (17), and 482 nm for nitrocefin (16). The initial velocity of the hydrolysis was measured and expressed in units of β-lactamase per milliliter of extract or per milligram (dry weight) of cells. One unit of β-lactamase is the amount of enzyme able to destroy 1 μmol of substrate per min at 37°C and at pH 7.0 (16). Against cefoxitin, ceftizoxime, and moxalactam the activity of the enzyme was too low to use the above procedure. Instead we used the millimolar absorbancy difference ($\Delta\epsilon$) (14) of those molecules to calculate the rate of hydrolysis observed in a 3-h incubation at 37°C. The $\Delta\epsilon$ values were, respectively, 7.38 mM^{-1} cm^{-1} at 265 nm, 7.03 mM^{-1} cm^{-1} at 250 nm, and 7.96 mM^{-1} cm^{-1} at 275 nm. A control without β-lactamase was also included to detect any spontaneous degradation of the antibiotic during the incubation period. For penicillins, the microiodometric assay of Sykes and Nordström (25) was used. The activity against cephaloridine was also measured by this technique for comparison purposes. The spectrophotometer used for this and for the inhibition studies was an Aminco DW-2 UV/VIS (American Instrument Co.) with an Aminco Midan microprocessor data analyzer (American Instrument Co.). We used 1-cm cuvettes and the temperature was controlled.

Isoelectric focusing. Isoelectric focusing was carried out on cylindrical gels in 200-μl micropipettes. The composition of the gel was as follows: acrylamide, 62.9 mg/ml; *N,N'*-methylenebisacrylamide, 3.5 mg/ml; Nonidet P-40 (Sigma Chemical Co.), 33 mg/ml; *N,N,N',N'*-tetramethylethylenediamide, 1.2 μl/ml; ammonium persulfate, 0.18 μg/ml; pH 3.5 to 10 ampholine (LKB, Bromma, Sweden), 3.3%. The gels were left in the micropipettes, and 50 μl of sample was applied on the cathode side of the gel. The current was applied at 400 V overnight and at 750 V for 1 h. The gels were flooded with 10^{-4} M nitrocefin and incubated at 37°C until bands of activity appeared. Two unused gels were cut and soaked in twice-distilled water for pH measurements. Bovine serum albumin was focused, fixed, and stained with Coomassie blue as a pI standard (pI, 4.85 to 4.90).

Inhibition studies. For inhibition studies, the initial velocity of degradation at different nitrocefin concentrations was measured at 37°C in the presence of various inhibitors. The following procedure was used: 0.5 ml of the inhibitor was incubated for 10 min with 0.5 ml of β-lactamase diluted in phosphate buffer without dithiothreitol to a final concentration able to destroy 10^{-4} M nitrocefin in 5 min. After incubation 1 ml of nitrocefin at 37°C was added, and the initial increase in absorbance at 482 nm against time was measured. The inhibitors studied were the following: cloxacillin (Beecham Laboratories) at a final concentration of 10^{-4} M; *p*-hydroxymercuribenzoate (PHMB; Sigma Chemical Co.), 10^{-4} M; clavulanic acid, 10^{-5} M; and cefoxitin, 10^{-5} M. The activities were measured against the following final concentrations of nitrocefin: 10^{-4}, 3×10^{-5}, 10^{-5}, 5×10^{-6}, 3×10^{-6}, and 10^{-6} M.

The V_{max} and K_m were calculated by iteration, using the Michaelis-Menten equation, and by minimizing the following equation: Σ {[speed of hydrolysis (observed)] − [speed of hydrolysis (calculated)]}^{2}. The speed of hydrolysis (observed) was defined as the speed of hydrolysis with different concentrations of nitrocefin, whereas the speed of hydrolysis (calculated) was the speed of hydrolysis determined with the Michaelis-Menten equation for the same concentrations of nitrocefin. For each curve a total of 700 equations were calculated, using a TRS 80 model III microcomputer (Tandy Corp., Fort Worth, Tex.) which provided more accurate K_m and V_{max} values than the Lineweaver-Burke method.

RESULTS

Detection of β-lactamase and susceptibility testing. Thirty-five of the 46 strains of *B. bivius* (76%) were β-lactamase positive with the nitrocefin slide test. Table 1 shows the in vitro activity of each agent against *B. bivius*. For all agents, the rank distribution of the MICs for the β-lactamase-positive strains was significantly different than that for the β-lactamase-negative strains ($P < 0.01$ for all agents; Mann-Whitney U rank test).

The effect of clavulanic acid on the susceptibility of β-lactamase-positive and -negative strains is shown also in Table 1. Clavulanic acid was used as a subinhibitory concentration of 0.75 μg/ml. All of our isolates of *B. bivius* were inhibited at either 8 or 16 μg of this agent alone per ml. The addition of clavulanic acid reduced the mean MICs of all agents, and for β-lactamase-positive strains the reduction was to the level of the mean MICs obtained for β-lactamase-negative strains. For cefoxitin, cephalothin, and penicillin G, clavulanic acid did not produce significant changes in the susceptibility of the β-lactamase-negative strains ($P > 0.05$; Wilcoxon signed rank test). For moxalactam there was significant change ($P < 0.05$; Wilcoxon signed rank test). For the other agents the effect of clavulanic acid could not be ascertained with these strains since they were already susceptible to the lowest concentration of the agent alone. These changes for the β-lactamase-negative strains were very small in comparison to the changes observed with β-lactamase-positive strains. In fact, for β-lactamase-negative strains, there was only a decrease of one dilution by clavulanic acid, when a decrease was observed. Of note is the reduction, by the addition of clavulanic acid, of the mean MICs of cefoxitin, cefotaxime, ceftizoxime, and moxalactam against β-lactamase-positive strains.

β-Lactamase assays. Crude extracts of β-lactamase were obtained from six different strains chosen because of their increasing resistance to all agents. Table 2 correlates the susceptibility of each of these strains to the various β-lactams and the activity of the corresponding crude extract against nitrocefin. For all agents, a higher activity of the crude extract against nitrocefin paralleled an increase in the MICs. Also, reduction of the MICs by the addition of clavulanic acid was more pronounced for strains with a high

TABLE 1. Antimicrobial susceptibility of *B. bivius* with and without the addition of clavulanic acid (0.75 μg/ml)

β-Lactam	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Mean MIC (μg/ml)[a]			
			Without clavulanic acid added		With clavulanic acid added	
			β-Lactamase positive	β-Lactamase negative	β-Lactamase positive	β-Lactamase negative
Cephaloridine	8	64	>19.6	≤0.01	≤0.09	≤0.09
Cephalothin	8	32	13.8	0.39	0.39	0.20
Penicillin G[b]	4	32	8.50	0.19	0.19	0.15
Moxalactam	4	16	5.49	0.44	0.44	0.27
Amoxicillin	2	16	4.00	≤0.06	≤0.06	≤0.06
Ceftazidime	2	8	3.24	1.00	ND[c]	ND
Cefamandole	2	8	2.98	0.94	ND	ND
Carbenicillin	1	16	2.47	≤0.17	ND	ND
Cefoxitin	1	4	1.43	0.32	0.32	0.25
Cefotaxime	0.5	8	1.17	≤0.06	≤0.06	≤0.06
Amoxicillin-clavulanic acid, 2:1	0.25	1	0.40	≤0.06	ND	ND
Ceftizoxime	0.06	1	0.17	≤0.02	≤0.02	≤0.015

[a] Logarithmic mean (base two).
[b] MICs given in units per milliliter.
[c] ND, Not done.

MICs. Also, reduction of the MICs by the addition of clavulanic acid was more pronounced for strains with a high hydrolytic activity against nitrocefin than for those with little or no hydrolytic activity.

The substrate profiles of the three most active extracts are shown in Table 3. Both penicillins and cephalosporins were hydrolyzed. Some activity against cefoxitin, ceftizoxime, and moxalactam was found in the 3-h assay: 0.1, 1.2, and 0.1 mU/mg (dry weight) against cefoxitin, ceftizoxime, and moxalactam for the *B. bivius* 1127 extract; 0.03, 0.6, and 0.05 mU/mg (dry weight) for the *B. bivius* 537 extract. The activities against cephaloridine were 0.12, 0.10, and 0.044 U per mg (dry weight) of cells, respectively, for the *B. bivius* 1127, 396, and 537 extracts.

Isoelectric focusing. A major band and a secondary band with much less activity were found with pI values of 5.6 and 7.0, respectively. In some extracts a streak band with a pI of about 4.8 to 5.0 was also detected.

Inhibition studies. Extracts of *B. bivius* 1127, 396, and 537 were used for inhibition studies. For comparison purposes a β-lactamase extract obtained from *B. fragilis* ATCC 25285 was included. Table 4 shows the V_{max} and the K_m of each extract against nitrocefin, with and without the addition of different inhibitors.

For the three *B. bivius* extracts, there was a major increase in the K_m with cefoxitin but little or no effect on the V_{max}, suggesting competitive inhibition of the β-lactamase with a K_i of about 3×10^{-5} M. With clavulanic acid there was a major decrease in the V_{max}, suggesting noncompetitive inhibition with a K_i of about 2×10^{-4} M. With cloxacillin and PHMB, there was little change in K_m or V_{max}, suggesting that these compounds are not effective inhibitors.

The four inhibitors were active against the *B. fragilis* ATCC 25285 extract. With cefoxitin, cloxacillin, and PHMB there was a major increase in the K_m with a K_i of 4×10^{-4} M for cefoxitin and a K_i of 10^{-4} M for cloxacillin and PHMB, suggesting a competitive inhibition of the enzyme by these compounds. With clavulanic acid, there was a large increase in the K_m and a decrease in the V_{max} with a K_i of 6×10^{-5} M, suggesting mixed noncompetitive inhibition.

DISCUSSION

β-Lactamase production is commonly found in *B. bivius*. Using a nitrocefin slide test, we detected β-lactamase in 76% of our strains. With a similar test Snydman et al. (21) found β-lactamase in 84% of 32 strains, whereas Tajima et al. (26) detected activity in 78% of 27 strains of *B. bivius*. The enzyme seems to play an important role in susceptibility to

TABLE 2. Correlation between susceptibility and β-lactamase activity against nitrocefin for six strains of *B. bivius* with increasing resistance to β-lactam agents

β-Lactam	MIC (μg/ml) for strains:					
	594	239	363	537	396	1127
Penicillin G[a]	0.12 (0)[b]	2 (4)	8 (6)	32 (7)	32 (7)	64 (7)
Amoxicillin	≤0.06 (—)[c]	1 (≥4)	4 (≥6)	16 (7)	16 (≥8)	32 (7)
Cephaloridine	≤0.06 (—)	4 (≥6)	16 (6)	64 (7)	64 (7)	64 (5)
Moxalactam	0.5 (1)	2 (3)	4 (5)	16 (4)	16 (4)	16 (4)
Cefotaxime	≤0.06 (—)	0.5 (≥3)	1 (≥4)	8 (≥7)	8 (≥7)	16 (7)
Cefoxitin	0.25 (0)	0.5 (2)	2 (3)	2 (2)	4 (4)	8 (4)
Ceftizoxime	0.03 (≥1)	0.12 (≥3)	0.12 (≥3)	1 (5)	1 (≥6)	4 (7)
Activity toward nitrocefin (mU/mg [dry wt] of cells)	1	9	20	80	90–160	330

[a] MICs given in units per milliliter.
[b] Number in parentheses indicates the reduction of susceptibility, in dilutions, with the addition of clavulanic acid (0.75 μg/ml).
[c] —, Could not be calculated: each MIC without clavulanic acid equal to or lower than the lowest dilution studied.

TABLE 3. Substrate profile of the *B. bivius* β-lactamase[a]

β-Lactam	Relative hydrolysis rate (%)[b] for strain:		
	1127	396	537
Penicillin G	26	ND[c]	ND
Amoxicillin	51	ND	ND
Cephaloridine	100	100	100
Cefotaxime	28	41	81
Cephalothin	27	25	55
Cefamandole	14	18	39
Ceftizoxime	1	1	1
Moxalactam	<1	<1	<1
Cefoxitin	<1	<1	<1
Nitrocefin	267	156	182

[a] Microiodometric assay for penicillins; spectrophotometric assay for cephalosporins.
[b] Velocity relative to cephaloridine.
[c] ND, Not done.

β-lactam agents. In our study, all strains lacking detectable β-lactamase were very susceptible to all β-lactam agents tested, with MICs of 2 μg/ml or less. In contrast, the β-lactamase-positive strains were inhibited at higher concentrations of all agents (Table 1).

Overall, the susceptibility pattern of our isolates were similar to that reported by other groups (1, 10, 11, 18, 21). In this study the most active agent in vitro was ceftizoxime, followed by a 2:1 combination of amoxicillin and clavulanic acid. Moxalactam had comparatively less in vitro activity against *B. bivius* than did cefotaxime and ceftizoxime.

The addition of clavulanic acid to β-lactams had interesting results. A major decrease in the MICs was seen only for β-lactamase-positive strains, which then became susceptible to all agents. This phenomenon cannot be attributed solely to an additive effect between the two agents since clavulanic acid caused only a minor increase in the susceptibility of β-lactamase-negative strains, and this change was significant only for moxalactam. This effect of clavulanic acid was also seen with cefotaxime, cefoxitin, ceftizoxime, and moxalactam. Moreover, the magnitude of the effect of clavulanic acid was related to the amount of β-lactamase found. There was also a relationship between the β-lactamase activity and the susceptibility to all agents (Table 2). The three strains producing the highest level of β-lactamase were found to be resistant to achievable blood levels of cephaloridine, cephalothin, and penicillin G (16 to 32 μg or U/ml), all of which were rapidly hydrolyzed. With other agents such as cefoxitin, ceftizoxime, and moxalactam these strains were also inhibited at the highest concentrations, but they were susceptible to achievable blood levels.

These observations suggest that the *B. bivius* β-lactamase may have some activity against cefoxitin, moxalactam, and ceftizoxime which can be blocked by clavulanic acid, although all of our strains were susceptible to those agents. Wüst and Wilkins (30) have previously reported that the addition of clavulanic acid increased the susceptibility of *B. bivius* to cefoxitin and they suggested then that this β-lactam could be hydrolyzed by this species. Our results, however, using the spectrophotometric assay, indicate that the enzymatic activity of the *B. bivius* extracts against cefoxitin is very low just as it is against ceftizoxime and moxalactam. This is in agreement with the fact that cefoxitin was found to be a potent competitive inhibitor of the enzyme.

Snydman et al. (21) reported clinical failures with penicillin G or cephalothin in postpartum endometrial infections with β-lactamase-producing *B. bivius*. Cefoxitin, moxalactam, and ceftazidime have been used with good results as single agents in similar infections (1, 5, 24). Whether the β-lactamase of *B. bivius* is able to slowly hydrolyze cefoxitin and other third-generation cephalosporins actually may be more of academic interest than clinical importance since, to our knowledge, clinical failures in infections involving *B. bivius* have not been reported so far with such agents.

Timewell et al. (28) recently classified the β-lactamases of the *Bacteroides* species into two different groups. Group I β-lactamases are inhibited by PHMB, cloxacillin, and clavulanic acid and are found in *B. fragilis*, *B. thetaiotaomicron*, *B. vulgatus*, and *B. uniformis*. Group II β-lactamases are inhibited by clavulanic acid but not by PHMB and cloxacillin and are found in *B. asaccharolyticus*, *B. melaninogenicus*, *B. bivius*, and *B. ovatus*. Our data on susceptibility to inhibitors confirm that the *B. bivius* β-lactamase belongs to group II. These enzymes are also different from those of group I because of their activity against both penicillins and cephalosporins.

With isoelectric focusing the major band observed, with a pI of 5.6, may be the same one that Timewell et al. (27) found at pI 5.7. As they reported, we observed in some gels a streak band with an approximate pI of 4.8 to 5.0. We also found a weak band with a pI of 7.0. Although it is possible that two different enzymes were present in our preparations, most of the activity focused at pI 5.6. Timewell et al. (27) suggested that the *B. bivius* β-lactamase may form a complex, possibly with a polysaccharide, because of the difficulty in focusing the enzyme. In our procedure, it is possible that Nonidet P-40, which is a nonionic detergent, helped to dissociate β-lactamase from this complex and so improved the focusing of the enzyme.

In summary, the β-lactamase of *B. bivius* seems to play a major role in the susceptibility of this species to β-lactam agents, the organism being resistant to those antibiotics that are rapidly hydrolyzed by the β-lactamase. The enzyme has

TABLE 4. V_{max} and K_m of the β-lactamases of *B. bivius* and *B. fragilis* in the presence of different inhibitors[a]

Inhibitor (M)	*B. bivius* 1127		*B. bivius* 396		*B. bivius* 537		*B. fragilis* ATCC 25285	
	V_{max} (U/ml of crude extract)	K_m (μM nitrocefin)	V_{max} (U/ml of crude extract)	K_m (μM nitrocefin)	V_{max} (U/ml of crude extract)	K_m (μM nitrocefin)	V_{max} (U/ml of crude extract)	K_m (μM nitrocefin)
None	6.88	13	0.77	4.3	1.37	4.1	0.23	7.7
Cefoxitin (10^{-5})	6.90	7,600	0.85	5,600	0.36	1,280	0.012	210
Clavulanic acid (10^{-5})	0.29	73	0.009	19.4	0.04	108	0.068	1,310
Cloxacillin (10^{-4})	5.93	29	0.54	41	0.87	34.4	0.83	7,080
PHMB (10^{-4})	4.43	8.8	1.50	19.4	1.75	14.8	0.21	5,800

[a] A 0.5-ml portion of β-lactamase was incubated with 0.5 ml of the inhibitor for 10 min at 37°C, and then 1 ml of nitrocefin was added to measure activity.

a mixed profile that is different from the profile of β-lactamases of species from the *B. fragilis* group. A low activity of the β-lactamase against cefoxitin, ceftizoxime, and moxalactam could be measured. This may account for the effect of clavulanic acid on the susceptibility of the β-lactamase-positive strains to those agents, but further studies are needed before firm conclusions can be drawn.

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LITERATURE CITED

1. **Blanco, J. D., R. S. Gibbs, P. Duff, Y. S. Castaneda, and P. J. St. Clair.** 1983. Randomized comparison of ceftazidime versus clindamycin-tobramycin in the treatment of obstetrical and gynecological infections. Antimicrob. Agents Chemother. **24**:500–504.
2. **Bourgault, A.-M., and J. E. Rosenblatt.** 1979. Characterization of anaerobic gram-negative bacilli by using rapid slide tests for β-lactamase production. J. Clin. Microbiol. **9**:654–656.
3. **Brook, I., L. Calhoun, and P. Yocum.** 1980. Beta-lactamase-producing isolates of *Bacteroides* species from children. Antimicrob. Agents Chemother. **18**:164–166.
4. **Gall, S. A., A. P. Kohan, O. M. Ayers, C. E. Hughes, W. A. Addison, and G. B. Hill.** 1981. Intravenous metronidazole or clindamycin with tobramycin for therapy of pelvic infections. Obstet. Gynecol. **57**:51–58.
5. **Gibbs, R. S., J. D. Blanco, P. Duff, Y. S. Castaneda, and P. J. St. Clair.** 1983. A double-blind, randomized comparison of moxalactam versus clindamycin-gentamicin in treatment of endomyometritis after cesarean section delivery. Am. J. Obstet. Gynecol. **146**:769–772.
6. **Gibbs, R. S., J. D. Blanco, P. J. St. Clair, and Y. S. Castaneda.** 1982. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J. Infect. Dis. **145**:1–8.
7. **Hirai, K., S. Iyobe, M. Inoue, and S. Mitsuhashi.** 1980. Purification and properties of a new β-lactamase from *Pseudomonas cepacia*. Antimicrob. Agents Chemother. **17**:335–358.
8. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobic laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
9. **Holdeman, L. V., and J. L. Johnson.** 1977. *Bacteroides disiens* sp. nov. and *Bacteroides bivius* sp. nov. from human clinical infections. Int. J. Syst. Bacteriol. **27**:337–345.
10. **Kaye, D., W. Kobasa, and K. Kaye.** 1980. Susceptibilities of anaerobic bacteria to cefoperazone and other antibiotics. Antimicrob. Agents Chemother. **17**:957–960.
11. **Kirby, B. D., W. L. George, V. L. Sutter, D. M. Citron, and S. M. Finegold.** 1980. Gram-negative anaerobic bacilli: their role in infection and patterns of susceptibility to antimicrobial agents. 1. Little-known *Bacteroides* species. Rev. Infect. Dis. **2**:914–951.
12. **Levison, M. E., L. C. Corman, E. R. Carrington, and D. Kaye.** 1977. Quantitative microflora of the vagina. Am. J. Obstet. Gynecol. **127**:80–85.
13. **Matthew, M., and R. W. Hedges.** 1976. Analytical isoelectric focusing of R-factor-determined β-lactamases: correlation with plasmid compatibility. J. Bacteriol. **125**:713–718.
14. **Minami, S., A. Yotsuji, M. Inoue, and S. Mitsuhashi.** 1980. Induction of β-lactamase by various β-lactam antibiotics in *Enterobacter cloacae*. Antimicrob. Agents Chemother. **18**:382–385.
15. **National Committee for Clinical Laboratory Standards.** 1979. Proposed reference dilution procedure for antimicrobic susceptibility testing of anaerobic bacteria. PSM-11. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
17. **O'Callaghan, C. H., P. W. Muggleton, and G. W. Ross.** 1968. Effects of β-lactamase from gram-negative organisms on cephalosporins and penicillins, p. 57–63. Antimicrob. Agents Chemother. 1968.
18. **Ohm-Smith, M. J., W. K. Hadley, and R. L. Sweet.** 1982. In vitro activity of new β-lactam antibiotics and other antimicrobial drugs against anaerobic isolates from obstetric and gynecological infections. Antimicrob. Agents Chemother. **22**:711–714.
19. **Olsson, B., C. E. Nord, and T. Wadström.** 1976. Formation of beta-lactamase in *Bacteroides fragilis*: cell-bound and extracellular activity. Antimicrob. Agents Chemother. **9**:727–735.
20. **Sautter, R. L., and W. J. Brown.** 1980. Sequential vaginal cultures from normal young women. J. Clin. Microbiol. **11**:479–484.
21. **Snydman, D. R., F. P. Tally, R. Knuppel, J. Landrigan, S. L. Gorbach, and J. G. Bartlett.** 1980. *Bacteroides bivius* and *Bacteroides disiens* in obstetrical patients: clinical findings and antimicrobial susceptibilities. J. Antimicrob. Chemother. **6**:519–525.
22. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. **9**:307–311.
23. **Sweet, R. L.** 1981. Treatment of mixed aerobic-anaerobic infections of the female genital tract. J. Antimicrob. Chemother. **8**(Suppl. D):105–114.
24. **Sweet, R. L., and W. J. Ledger.** 1979. Cefoxitin: single-agent treatment of mixed aerobic-anaerobic pelvic infections. Obstet. Gynecol. **54**:193–198.
25. **Sykes, R. B., and K. Nordström.** 1972. Microiodometric determination of β-lactamase activity. Antimicrob. Agents Chemother. **1**:94–99.
26. **Tajima, M., K. Sawa, and K. Watanabe.** 1983. The β-lactamases of genus *Bacteroides*. J. Antibiot. **36**:423–428.
27. **Timewell, R. M., I. Phillips, J. Söderholm, and C. E. Nord.** 1981. Isoelectric focusing of *Bacteroides melaninogenicus* group β-lactamases. Antimicrob. Agents Chemother. **19**:700–704.
28. **Timewell, R., E. Taylor, and I. Phillips.** 1981. The β-lactamases of *Bacteroides* species. J. Antimicrob. Chemother. **7**:137–146.
29. **Wilkins, T. D., and S. Chalgren.** 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. **10**:926–928.
30. **Wüst, J., and T. D. Wilkins.** 1978. Effect of clavulanic acid on anaerobic bacteria resistant to beta-lactam antibiotics. Antimicrob. Agents Chemother. **13**:130–133.