An investigation of β -lactamases from clinical isolates of *Bacteroides* species

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Summary. Among a group of 116 clinically significant isolates of *Bacteroides* spp., 24 exhibited β -lactamase activity greater than the basal level characteristic of most *Bacteroides* strains. Investigation of specific enzyme activity, iso-electric point and enzyme inhibition profiles revealed that the β -lactamases involved could be divided into four groups, some showing similarity to those described in previous studies. Seven of the enzymes were able to hydrolyse cefoxitin, latamoxef or imipenem, and eight enzymes degraded penicillin in the presence of clavulanic acid. Five strains showed reduced susceptibility to cefoxitin, latamoxef or imipenem which was not associated with β -lactamase activity.

Introduction

The production of β -lactamase is the major factor in the resistance of *Bacteroides* spp. to β -lactam antibiotics. Most *Bacteroides* strains exhibit low-level chromosomally mediated β -lactamase activity that affects benzylpenicillin and many other penicillins, although the enzyme preferentially hydrolyses cephalosporins.¹ A substantial number of clinical isolates show raised levels of β -lactamase activity.^{2–4} Some of these enzymes inactivate β -lactamase-stable compounds, including cefoxitin, latamoxef and imipenem, and are insusceptible to inhibition by β -lactamase inhibitors, such as clavulanic acid or sulbactam.^{4–8}

Attempts have been made to classify the β -lactamases produced by *Bacteroides* spp. according to their substrate profiles, susceptibility to various enzyme inhibitors, iso-electric point, specific enzyme activity and rate of hydrolysis of susceptible substrates.^{3, 7} In a previous communication,⁴ we used such methods to examine the β -lactamases of a group of *Bacteroides* strains isolated from clinical specimens in Nottingham, and suggested that those strains exhibiting enhanced levels of enzyme activity could be grouped according to their specific cephalosporinase activity.

We have now examined a further group of clinical isolates to assess the prevalence of the various enzyme types and to try to shed further light on the characterisation of these enzymes.

Materials and methods

Bacterial strains

One hundred and sixteen clinically significant isolates of *Bacteroides* spp. were collected randomly

from specimens submitted to Nottingham Public Health Laboratory and maintained in skimmed milk at -70°C . They were identified by carbohydrate fermentation tests and, if these tests yielded equivocal results, by the ATB 32A system (API Products, Basingstoke, Hants). *B. fragilis* NCTC 9344 was used as a control strain.

Antibiotics

Solutions of benzylpenicillin (Glaxo Laboratories Ltd), clavulanic acid (Beecham Research Laboratories Ltd), cefoxitin (Merck, Sharp and Dohme Ltd) and latamoxef (Eli Lilly and Co. Ltd) were freshly prepared as required in sterile distilled water. Appropriate concentrations of imipenem (Merck, Sharp and Dohme Ltd) were dissolved in 0.01M phosphate buffer, pH 7.0, according to the manufacturer's instructions.

Antibiotic titrations

Minimum inhibitory concentrations (MICs) were determined by the agar incorporation method. Antibiotics in suitable two-fold serial concentrations were incorporated in Brain Heart Infusion Agar supplemented with yeast extract 5 g/L, haemin 5 mg/L, menadione 1 mg/L and lysed horse blood 5%. An inoculum of $c. 10^6$ organisms was delivered on to the agar surface with a multipoint inoculator (Denley Instruments, Billingshurst, Sussex). The lowest antibiotic concentration that completely inhibited growth after incubation for 48 h at 37°C , in an anaerobic cabinet, was taken as the MIC.

Semi-quantification of β -lactamase in intact bacteria

Penicillinase. The method of Escamilla⁹ was used. In this test, a pH change occurring as a result of

hydrolysis of benzylpenicillin is assessed by a colour change in a phenol red indicator.

Cephalosporinase. The hydrolysis of nitrocefin (250 mg/L), which results in a colour change from yellow to red, was used as described by Aldridge *et al.*¹⁰

In both tests, several colonies from overnight cultures of organisms grown on supplemented Brain-Heart infusion agar were used and the results were assessed visually over a 1-h period.

Preparation of crude β -lactamase extracts

Bacterial growth was harvested from Blood Agar plate cultures (Blood Agar Base, Oxoid, with horse blood 7%) after incubation for 48 h in an anaerobic cabinet. The bacteria were suspended in a tube containing 4 ml of phosphate buffer (0.02 M; pH 7.0) in a beaker of iced water. The bacterial suspensions were then subjected to six 30-s bursts of sonication, on ice, in a Soniprep 150 ultrasonicator (MSE Scientific Instruments, Crawley, Sussex). The extracts were centrifuged at 4000 rpm for 30 min at 4°C (Coolspin; MSE) and the supernate (crude extract) was divided into four portions and stored at -70°C.¹¹

Determination of specific activity

The method used was based on that of O'Callaghan *et al.*¹² The rate of hydrolysis of nitrocefin (1.9 ml of a 51.6 mg/L solution in phosphate buffer, pH 7.0) by 0.1 ml of crude enzyme extract was measured over 3 min at 37°C and 482 nm in a spectrophotometer (UV-160A; Shimadzu, Kyoto, Japan). Extracts achieving maximum hydrolysis in under 3 min were diluted appropriately in phosphate buffer and re-tested. The total soluble protein content of the crude extracts was measured by the Sigma Protein Assay Kit. Specific enzyme activity was calculated as the amount (μ mol) of nitrocefin hydrolysed/min/mg of protein.

Iso-electric focusing

Iso-electric focusing of crude β -lactamase extracts was done on cellulose acetate membranes as previously described.¹³

Inhibition profiles

Enzyme inhibition analysis was performed in a centrifugal fast analyser (Centrifichem 400; Union Carbide, Terrytown, New York, USA) by following the colour change induced in nitrocefin in the presence of serial 10-fold concentrations (range 0.1–100 μ M) of imipenem, latamoxef, cefoxitin, clavulanic acid, sulbactam (Pfizer Ltd), cloxacillin (Beecham Research Laboratories Ltd.) and *p*-chloromercuribenzoate (pCMB) (Sigma) as described by Eley and Greenwood.⁷ The activities of the crude β -lactamase extracts

were standardised before testing by appropriate dilution with 0.02 M phosphate buffer.

High pressure liquid chromatography (HPLC)

The kinetics of hydrolysis of latamoxef, cefoxitin, imipenem and the combination of benzylpenicillin and clavulanic acid (4 mg/L) was investigated by HPLC; 0.2 ml of enzyme extract, concentrated five times by freeze drying, was mixed with 0.8 ml of antibiotic solution to give a final concentration of 50 mg/L and incubated at 37°C. Samples were removed for HPLC analysis at 0, 2, 4 and 20 h. Antibiotic controls, in which phosphate buffer was substituted for enzyme extract, were examined in parallel. A Hypersil 50DS column (HPLC Technology, Macclesfield, Cheshire) was used. The mobile phase for analysis of benzylpenicillin and cefoxitin was methanol:water 40:60 with detection at wavelengths of 227 nm and 235 nm, respectively. For latamoxef, methanol:water 25:75 was used with detection at 250 nm, and for imipenem, methanol:water 20:80 with detection at 300 nm. Phosphoric acid 1% was included as a mobile phase modifier throughout these experiments.

Results

Identification of isolates

The 116 isolates were identified as: *B. fragilis* (69), *B. distasonis* (16), *B. ovatus* (13), *B. thetaiotaomicron* (6), *B. melaninogenicus* (4), *B. vulgatus* (4), *B. bivia* (3) and *B. buccae* (1).

Semi-quantification of enzyme activity

Of the 116 isolates, 28 failed to induce a colour change in nitrocefin within 1 h, 64 produced a weak reaction, and 24 caused a complete colour change. The latter strains (14 *B. fragilis*, 5 *B. ovatus*, 2 *B. bivia*, 1 *B. thetaiotaomicron*, 1 *B. distasonis* and 1 *B. buccae*) were selected for further characterisation of their β -lactamases. Fifteen of the 24 strains that produced enhanced levels of cephalosporinase also gave positive reactions in the Escamilla test for penicillinase.

Antibiotic titrations

MICs of the antibiotics for all 116 test strains are shown in table I. Imipenem and the combination of benzylpenicillin with clavulanic acid were more active than cefoxitin, latamoxef or benzylpenicillin alone. Eleven of the 24 strains with raised β -lactamase levels exhibited increased resistance to benzylpenicillin (MIC > 32 mg/L). Clavulanic acid 4 mg/L reduced the MIC of benzylpenicillin more than two-fold with all but three of the 24 test strains; in all but six cases the susceptibility to benzylpenicillin was reduced to

Table I. Susceptibilities of *Bacteroides* strains to selected β-lactam antibiotics

Antibiotic	Minimum inhibitory concentration (mg/L)		
	Range	MIC50	MIC90
Benzylpenicillin	0.5–> 64	16	32
Benzylpenicillin + clavulanic acid	0.06–16	0.25	2
Cefoxitin	0.25–> 128	16	32
Latamoxef	0.25–> 128	1	16
Imipenem	0.03–4	0.5	2

MIC50 and MIC90, concentrations inhibiting 50% and 90% of strains, respectively.

< 2 mg/L in the presence of clavulanic acid 4 mg/L (table II). The strains displayed a wide range of susceptibilities to the other test antibiotics. Four strains, *B. fragilis* R212 and R208, *B. ovatus* R215 and *B. thetaiotaomicron* R233 exhibited markedly reduced susceptibility to cefoxitin, latamoxef and imipenem.

Specific enzyme activity

Specific cephalosporinase activity (μmol of nitrocefin degraded/min/mg of protein) of the crude enzyme extracts ranged from 0.01 to 1.33 (table III). All the strains for which the specific activity was ≥ 0.1 exhibited high penicillinase activity as determined by the whole-cell semi-quantitative test, whereas many

of the β-lactamases with lower specific activity lacked penicillinase activity.

Iso-electric focusing

Crude extracts of *B. fragilis* isolates yielded a single iso-electric point between 4.9 and 5.3 (table III). No band was detected by iso-electric focusing of extracts of three other *B. fragilis* strains (R240, R249, R251) despite repeated testing. Enzymes from the five *B. ovatus* strains displayed a wide range of pI values (4.5–6.7). The two *B. bivius* strains displayed high pI values (> 6.0).

Inhibition profiles

Only five of the 24 strains (*B. fragilis* R106, R216 and R226, *B. ovatus* R111 and R112) produced β-lactamases that were at least partially susceptible to all the inhibitors tested; a further seven were susceptible to all but pCMB (table IV). The concentrations of inhibitors required to achieve inhibition of the individual enzymes varied considerably. The β-lactamase from *B. fragilis* R186 was not inhibited by any of the agents tested and three *B. fragilis* strains (R240, R249 and R251) displayed very low levels of inhibition (table IV). There were only three profiles common to more than one strain: those shown by *B. fragilis* R130 and R152; *B. buccae* R147 and *B. bivius* R160; and *B. ovatus* R137 and *B. fragilis* R141.

Table II. Antibiotic susceptibility (MIC mg/L) of 24 strains of *Bacteroides* that exhibited increased β-lactamase activity

Bacterial strain	Minimum inhibitory concentration (mg/L)				
	benzyl penicillin	benzylpenicillin + clavulanic acid	cefoxitin	latamoxef	imipenem
<i>B. fragilis</i> R106	32	0.5	16	1	0.5
<i>B. fragilis</i> R130	32	0.5	8	1	0.25
<i>B. fragilis</i> R249	4	2	16	0.5	1
<i>B. fragilis</i> R251	16	2	32	4	2
<i>B. fragilis</i> R186	8	4	32	1	2
<i>B. fragilis</i> R240	8	4	32	1	4
<i>B. buccae</i> R147	16	<0.12	<0.5	0.5	<0.03
<i>B. ovatus</i> R137	16	0.5	8	1	0.5
<i>B. ovatus</i> R111	16	0.25	4	1	0.5
<i>B. bivius</i> R160	4	<0.12	4	4	0.06
<i>B. distasonis</i> R118	32	1	32	16	2
<i>B. fragilis</i> R208	> 64	1	32	> 128	4
<i>B. fragilis</i> R141	16	0.25	16	0.5	0.06
<i>B. bivius</i> R143	16	0.25	4	4	0.03
<i>B. fragilis</i> R126	> 64	1	16	16	0.5
<i>B. ovatus</i> R215	> 64	0.5	64	> 128	1
<i>B. fragilis</i> R152	> 64	<0.12	8	8	0.12
<i>B. ovatus</i> R112	> 64	0.25	16	16	0.5
<i>B. fragilis</i> R226	> 64	0.5	8	16	1
<i>B. fragilis</i> R216	> 64	1	8	8	1
<i>B. ovatus</i> R102	> 64	1	32	32	0.5
<i>B. fragilis</i> R212	> 64	4	128	> 128	2
<i>B. fragilis</i> R134	> 64	0.25	16	16	0.25
<i>B. thetaiotaomicron</i> R233	> 64	16	> 128	> 128	4
<i>B. fragilis</i> NCTC9344*	16	0.25	8	0.25	0.12

* Data from reference 7, included for comparison.

Table III. Characterisation of β -lactamases from 24 strains of *Bacteroides* that exhibited increased β -lactamase activity

Bacterial strain	β -lactamase activity		Specific activity*	Iso-electric point
	cephalosporinase	penicillinase		
<i>B. fragilis</i> R106	+++	—	0.01	5.3
<i>B. fragilis</i> R130	++	—	0.01	5.0
<i>B. fragilis</i> R249	++	—	0.01	ND
<i>B. fragilis</i> R251	+	++	0.01	ND
<i>B. fragilis</i> R186	+++	—	0.02	4.9
<i>B. fragilis</i> R240	++	++	0.02	ND
<i>B. buccae</i> R147	++	++	0.03	5.2
<i>B. ovatus</i> R137	++	++	0.03	4.5
<i>B. ovatus</i> R111	+++	+	0.05	4.8
<i>B. bivius</i> R160	+++	—	0.05	6.1
<i>B. distasonis</i> R118	++	+	0.07	4.6, 5.0
<i>B. fragilis</i> R208	++	—	0.08	5.0
<i>B. fragilis</i> R141	++	—	0.09	5.0
<i>B. bivius</i> R143	+++	+++	0.10	6.4
<i>B. fragilis</i> R126	+++	+++	0.15	5.0
<i>B. ovatus</i> R215	++	+++	0.16	5.0, 6.7
<i>B. fragilis</i> R152	+++	+++	0.21	5.2
<i>B. ovatus</i> R112	+++	+++	0.44	5.1
<i>B. fragilis</i> R226	+++	+++	0.68	4.9
<i>B. fragilis</i> R216	+++	+++	0.71	4.9
<i>B. ovatus</i> R102	+++	+++	0.87	5.0
<i>B. fragilis</i> R212	+++	+++	0.88	5.1
<i>B. fragilis</i> R134	+++	+++	0.93	4.9
<i>B. thetaiotaomicron</i> R233	+++	+++	1.33	4.6
<i>B. fragilis</i> NCTC9344	±	—	0.03	5.1

+ — + + +, arbitrary levels of enzyme activity assessed visually according to the speed and completeness of the colour change. Isolates that exhibited basal levels of cephalosporinase activity, scored as ± after 1h, are omitted from this table (results for *B. fragilis* NCTC 9344⁷ included for comparison).

* Nitrocefin μ mol degraded/min/mg of protein.

ND, not detected.

Table IV. Inhibitor profiles of β -lactamases of strains listed in tables II and III

Bacterial strain	IC ₅₀ (μ M)						
	pcmb	imipenem	latamoxef	cefoxitin	clavulanic acid	sulbactam	cloxacillin
<i>B. fragilis</i> R106	10	<0.1	<0.1	<0.1	<0.1	<0.1	10
<i>B. fragilis</i> R130	>100	<0.1	<0.1	<0.1	<0.1	1	100
<i>B. fragilis</i> R249	10	>100	>100	>100	>100	>100	100
<i>B. fragilis</i> R251	100	>100	>100	>100	>100	>100	100
<i>B. fragilis</i> R186	>100	>100	>100	>100	>100	>100	>100
<i>B. fragilis</i> R240	100	>100	>100	>100	>100	>100	>100
<i>B. buccae</i> R147	>100	<0.1	<0.1	<0.1	<0.1	1	>100
<i>B. ovatus</i> R137	>100	<0.1	<0.1	<0.1	<0.1	<0.1	100
<i>B. ovatus</i> R111	10	<0.1	<0.1	<0.1	<0.1	<0.1	100
<i>B. bivius</i> R160	>100	<0.1	<0.1	<0.1	<0.1	1	>100
<i>R. distasonis</i> R118	>100	<0.1	<0.1	<0.1	1	10	>100
<i>B. fragilis</i> R208	>100	<0.1	100	1	10	10	>100
<i>B. fragilis</i> R141	>100	<0.1	<0.1	<0.1	<0.1	<0.1	100
<i>B. bivius</i> R143	>100	<0.1	100	1	<0.1	10	>100
<i>B. fragilis</i> R126	>100	<0.1	1	<0.1	<0.1	<0.1	100
<i>B. ovatus</i> R215	>100	<0.1	10	1	1	1	100
<i>B. fragilis</i> R152	>100	<0.1	<0.1	<0.1	<0.1	1	100
<i>B. ovatus</i> R112	100	<0.1	<0.1	<0.1	<0.1	<0.1	100
<i>B. fragilis</i> R226	1	<0.1	<0.1	<0.1	<0.1	<0.1	10
<i>B. fragilis</i> R216	1	<0.1	<0.1	<0.1	<0.1	<0.1	1
<i>B. ovatus</i> R102	100	<0.1	<0.1	<0.1	<0.1	>100	>100
<i>B. fragilis</i> R212	>100	<0.1	100	1	1	1	>100
<i>B. fragilis</i> R134	>100	<0.1	<0.1	1	<0.1	<0.1	100
<i>B. thetaiotaomicron</i> R233	>100	<0.1	100	1	1	10	>100

IC₅₀, lowest concentration of inhibitor producing > 50% inhibition; pcmb, *p*-chloromercuribenzoate.

HPLC

Figs. 1–4 show the rates of hydrolysis of benzylpenicillin (in the presence of clavulanic acid), cefoxitin, latamoxef and imipenem for those strains in which breakdown was detected by HPLC. In the presence of clavulanic acid, benzylpenicillin was rapidly degraded by enzymes from *B. fragilis* strains R186, R212, R240, R249 and R251 and *B. thetaiotaomicron* R233; enzymes from *B. bivius* R143 and *B. ovatus* R215 hydrolysed penicillin more slowly (fig. 1). All these strains, with the exception of *B. bivius* R143, also broke down cefoxitin and latamoxef (figs. 2 and 3); latamoxef was hydrolysed more rapidly than cefoxitin in all cases. Only four of the test strains, R186, R240, R249 and R251, were active against imipenem; all hydrolysed the antibiotic rapidly, but a small amount of the

Fig. 1. Hydrolysis of benzylpenicillin in the presence of clavulanic acid 4 mg/L by enzyme extracts from *Bacteroides* strains; —○—, control; —●—, R212, R233, R240, R251; —△—, R215; —□—, R249; —■—, R186; —▲—, R143.

Fig. 2. Hydrolysis of cefoxitin by enzyme extracts from *Bacteroides* strains; —○—, control; —▲—, R212, R233; —△—, R215; —□—, R249; —■—, R186; —□—, R240; —●—, R251.

Fig. 3. Hydrolysis of latamoxef by enzyme extracts from *Bacteroides* strains; —○—, control; —●—, R212, R233, R251; —△—, R215; —□—, R249; —■—, R186; —□—, R240.

Fig. 4. Hydrolysis of imipenem by enzyme extracts from *Bacteroides* strains; —○—, control; —□—, R249; —■—, R186; —□—, R240; —●—, R251.

compound was still detectable after overnight incubation (fig. 4).

Discussion

Some unusual properties of the β -lactamases of *Bacteroides* spp. have been revealed in previous studies,^{3,4,7} but the characterisation and classification of these enzymes remain ill-defined.

In the present study of 116 clinically significant isolates of *Bacteroides*, 24 produced levels of cephalosporinase activity greater than the basal level typical of most *Bacteroides* strains, and two-thirds of these also exhibited the capacity to hydrolyse penicillinase as judged by the phenol red test.⁹ Fourteen of these 24 strains were identified as *B. fragilis*, and six of the

seven strains that failed to give a positive result in the test for penicillinase activity belonged to this species. In contrast to a previous study of 78 clinical isolates of the *B. fragilis* group, in which all but one of 13 "high β -lactamase" producers were identified as *B. fragilis sensu stricto*,⁴ seven isolates were identified as other members of the *B. fragilis* group, including five *B. ovatus* strains.

As in the earlier study,⁴ imipenem and the combination of benzylpenicillin with clavulanic acid were much more active than cefoxitin, latamoxef or benzylpenicillin alone, as judged by conventional MIC titration. The overall level of resistance remained low, although there seems to have been a slight increase in the prevalence of resistance to cefoxitin and imipenem in the intervening 5 years: for nine strains (8%), the MIC of cefoxitin was ≥ 32 mg/L; five of these strains (4%) were also resistant to latamoxef (MIC ≥ 32 mg/L). A total of seven strains (6%) displayed reduced susceptibility to imipenem (MIC ≥ 2 mg/L). The proportions of strains in the previous study exhibiting this level of resistance were cefoxitin (4%), latamoxef (6%) and imipenem (4%). Among the present strains, seven (6%) were able to hydrolyse cefoxitin, latamoxef or imipenem; 4% of strains in the earlier study displayed this property.

The categorisation of the β -lactamases of *Bacteroides* spp. is problematic. Thus, enzymes classified on the basis of their differential ability to hydrolyse β -lactamase-stable compounds such as cefoxitin, latamoxef, imipenem and clavulanic acid often exhibit heterogeneous properties according to other measures of activity, such as inhibition profiles and specific enzyme activity.

Use of specific activity as a differential feature presents particular problems, because the conditions of growth of the organisms, as well as the methods used for preparation, concentration and storage of the enzyme extracts, may profoundly affect the results.¹¹ These factors were carefully standardised in the present investigation, but variability originating in methodological differences makes comparison with the results of other studies difficult.

Despite the problem of reconciling so many heterogeneous properties, certain tentative groupings can be perceived among the strains described in the present study and others previously reported (table V).^{4,7}

In the first group are the enzymes produced by *B. fragilis* strains R186, R240, R249 and R251, which appear to have broadly similar characteristics: low specific activity; insusceptibility to inhibition by β -lactamase inhibitors, including clavulanic acid; relative resistance to imipenem; and the ability to degrade imipenem, latamoxef and, more slowly, cefoxitin. These properties are similar to those previously described in *B. fragilis* strains, 57, 97 and 119,^{4,7} although the failure to detect iso-electric focusing bands in *B. fragilis* strains R240, R249 and R251 is anomalous.

The second group is represented by *B. fragilis* R212, *B. ovatus* R215 and *B. thetaiotaomicron* R233, all of which exhibited resistance to benzylpenicillin, cefoxitin and latamoxef and reduced susceptibility to imipenem; they also displayed intermediate, or high specific activity. Enzymes from these strains showed similar (but not identical) inhibition profiles and hydrolysed β -lactamase-stable compounds other than imipenem. These enzymes are clearly not identical, since their iso-electric points are diverse as would be expected of enzymes from these different species. The overall behaviour is similar to that of enzymes produced by *B. thetaiotaomicron* 0456 and *B. distasonis* R939, described previously.⁷

A third group, represented by two strains, *B. distasonis* R118 and *B. fragilis* R208, exhibited reduced susceptibility to cefoxitin, latamoxef and imipenem, but did not hydrolyse these antibiotics as judged by HPLC. The enzymes displayed variable inhibition profiles and intermediate levels of specific activity. Other strains have been described that appear to be resistant to β -lactamase-stable compounds although they do not hydrolyse them. Such strains seem to belong to a heterogeneous group which may possess other mechanisms of resistance¹⁴ and these will be the subject of further study.

Most of the strains encountered in this, and a previous study,⁴ retain a high degree of susceptibility to cefoxitin, latamoxef and imipenem. Generally, the enzymes are fully susceptible to β -lactamase inhibitors and clavulanic acid fully restores the activity of benzylpenicillin. These strains produce enzymes with a wide range of specific activities that have no hydrolytic activity against β -lactamase-stable compounds. These strains were identified as enhanced β -

Table V. Characteristics of four types of β -lactamases from 24 *Bacteroides* strains

Type	MIC (mg/L) of				Inhibitors of β -lactamase	Activity of benzylpenicillin restored by clavulanic acid	Hydrolysis of			
	benzyl penicillin	cefoxitin	latamoxef	imipenem			benzyl penicillin*	cefoxitin	latamoxef	imipenem
Type 1	4-16	16-32	0.5-4	1-4	Resistant	No	Yes	Yes (slow)	Yes	Yes
Type 2	> 64	> 32	> 128	1-4	Intermediate	Partial	Yes	Yes	Yes	No
Type 3	> 16	32	> 8	2-4	Variable	Yes	No	No	No	No
Type 4	4->64	4-32	0.5-32	≤ 1	Susceptible	Yes	No	No	No	No

* In the presence of clavulanic acid.

lactamase-producers by the nitrocefin screening procedure and it is probable that they are strains that produce greater than normal amounts of the cephalosporinase that most *B. fragilis* group strains are known to produce.

The enzyme produced by *B. bivia* R143 did not conform to any of the categories described above, notably by slowly hydrolysing benzylpenicillin in the presence of clavulanic acid. In other respects its behaviour was similar to that of the other *B. bivia* strain R160, although it was considerably less susceptible to inhibitors of β -lactamase activity.

The strains that we have examined were selected on

the grounds of their involvement in some pathogenic process and most of the species encountered belonged to the *B. fragilis* group. Others have described β -lactamases with heterogeneous properties from non-*B. fragilis* group species¹⁵ and it is clear that the β -lactamases of anaerobic gram-negative rods are much more variable than those of enterobacteria.^{16, 17} Fortunately, the number of strains that exhibit enzymic or other mechanisms of resistance to β -lactam antibiotics appears presently to be quite small, but there is no guarantee that this situation will continue to prevail under the selective pressure of antibiotic usage.

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