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β-Lactamase Production and Susceptibilities to Amoxicillin, Amoxicillin-Clavulanate, Ticarcillin, Ticarcillin-Clavulanate, Cefoxitin, Imipenem, and Metronidazole of 320 Non-*Bacteroides fragilis Bacteroides* Isolates and 129 Fusobacteria from 28 U.S. Centers

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β-Lactamase production (nitrocefin disk method) and agar dilution susceptibility of amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, cefoxitin, imipenem, and metronidazole were determined for 320 *Bacteroides* species (not *Bacteroides fragilis* group) and 129 fusobacteria from 28 U.S. centers. Overall, 64.7% of *Bacteroides* species and 41.1% of fusobacteria were β-lactamase positive. Among the *Bacteroides* species, positivity rates were highest for *B. bivius* (85.0%), followed by *B. splanchnicus* (83.3%), *B. eggerthii* (77.8%), and *B. oralis* (77.1%); 54.5% of black-pigmented *Bacteroides* species were β-lactamase positive. Among the fusobacteria, *Fusobacterium mortiferum* showed the highest rate of β-lactamase positivity (76.9%). MICs of amoxicillin (128 μg/ml) and ticarcillin (64 μg/ml) for 90% of all β-lactamase-positive strains were reduced to 4 and 2 μg/ml, respectively, with the addition of clavulanate. MICs of amoxicillin and ticarcillin for 90% of all β-lactamase-negative strains were 1 and 4 μg/ml, respectively, and ≥98.4% of the strains were susceptible to the β-lactams tested. Of the β-lactamase-producing strains, 45.9% were susceptible to amoxicillin at ≤4 μg/ml and 93.4% were susceptible to ticarcillin at ≤64 μg/ml; the addition of clavulanate raised the rates to 90.4 and 100%, respectively. All strains were susceptible to cefoxitin, imipenem, and metronidazole. The activity of amoxicillin against 29 β-lactamase-producing strains (10 *Bacteroides* species and 19 fusobacteria) was not enhanced by the addition of clavulanate; however, 82.7% of these strains were susceptible to amoxicillin, and all were susceptible to ticarcillin. Although β-lactamase positivity is on the increase in non-*B. fragilis* group *Bacteroides* species and fusobacteria, amoxicillin-clavulanate, ticarcillin, cefoxitin, imipenem, and metronidazole should be suitable for the treatment of infections with these strains. The addition of clavulanate does not appreciably improve the efficacy of ticarcillin against these organisms.

Gram-negative anaerobic bacteria are established as important pathogens, especially in patients with weakened host defenses such as those caused by malignancy, malnutrition, debilitation, trauma, and surgery (2, 26). Although the *Bacteroides fragilis* group comprises the most important group of anaerobic gram-negative pathogens, infections with other *Bacteroides* species and fusobacteria are increasingly encountered (2, 11, 16, 17, 26, 28). As the susceptibility patterns of these organism groups has been assumed to be relatively stable, routine susceptibility testing has not been regarded as necessary (24, 27, 28). The problem is complicated by the difficult nature of anaerobe susceptibility testing in general and, in particular, of the testing of fastidious and more slowly growing organisms such as non-*B. fragilis* group *Bacteroides* species and fusobacteria (7, 24, 28).

In recent years, however, the antimicrobial susceptibility spectrum of non-*B. fragilis* group anaerobic gram-negative rods has undergone gradual change (2, 28). β-Lactamase production has been reported for several non-*B. fragilis* group *Bacteroides* species, including *B. bivius*, the black-pigmented group, *B. oris* or *B. buccae*, *B. oralis*, *B. splanchnicus*, *B. coagulans*, *B. ureolyticus*, *B. multiacidus* (reclassified as *Mitsuokella multiacidus*), and *B. hypermegas* (reclassified as *Megamonas hypermegas*) (6, 7, 9, 14, 15, 18, 19, 26, 29, 30). β-Lactamase positivity has also recently been described in fusobacteria, notably, *Fusobacterium nucleatum* (5, 31). To monitor current patterns of susceptibilities of organisms such as non-*B. fragilis* group anaerobic gram-negative bacilli, periodic countrywide surveys should be performed by a standardized technique and organisms should be isolated 1 or 2 years prior to the evaluation (5). In this study we used standardized methods to examine the β-lactamase positivity rates and susceptibilities to amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, cefoxitin, imipenem, and metronidazole of 320 non-*B. fragilis* group *Bacteroides* species and 129 fusobacteria isolated within 2 years of the survey from 28 centers around the United States, chosen to yield as wide a geographic spread as possible.

MATERIALS AND METHODS

Bacterial strains. Organisms were obtained from Hershey Medical Center, University Hospitals of Cleveland, and the centers listed in Acknowledgments, transported to Hershey Medical Center, and stored at −70°C in sterile defibrinated sheep blood. Strains were all recent clinical in-patient isolates from normally sterile sites and were obtained from blood cultures and material from orofacial, pulmonary, intra-abdominal, upper genital, and other infections. The majority of the strains were obtained prior to the institution of antimicrobial therapy. Prior to being tested, the organisms

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TABLE 1. Results of nitrocefin testing for β-lactamase production by 449 non-*B. fragilis Bacteroides* species and fusobacteria

Species	No. tested	No. (%) positive
Bacteroides	320	207 (64.7)
B. oris or *B. buccae*	16	6 (37.5)
B. splanchnicus	6	5 (83.3)
B. melaninogenicus	31	20 (64.5)
B. asaccharolyticus	8	1 (12.5)
B. intermedius	27	14 (51.9)
B. loeschei	8	5 (62.5)
B. oralis	35	27 (77.1)
B. eggerthii	9	7 (77.8)
B. bivius	100	85 (85.0)
B. disiens	24	15 (62.5)
B. ureolyticus	27	6 (22.2)
B. capillosus	22	13 (59.1)
Other[a]	7	3 (42.9)
Fusobacterium	129	53 (41.1)
F. nucleatum	42	9 (21.4)
F. necrophorum	22	5 (22.7)
F. mortiferum	26	20 (76.9)
F. varium	34	17 (50.0)
Other[b]	5	2 (40.0)
All isolates	449	260 (57.9)

[a] *B. buccalis* (*n* = 1), *B. gingivalis* (*n* = 2), *B. denticola* (*n* = 1), and *B. gracilis* (*n* = 3).
[b] *F. gonidiaformans* (*n* = 3) and *F. naviforme* (*n* = 2).

were checked for purity by subculturing on brucella blood agar and either kanamycin-vancomycin plates for *Bacteroides* species or josamycin-neomycin plates for fusobacteria (2, 26). Purity was also checked by Gram staining. Identification of organisms was done by standard methods (2, 26).

β-Lactamase testing. Nitrocefin disks (Cefinase disks; BBL Microbiology Systems, Cockeysville, Md.) were inoculated with a small portion of growth from brucella blood agar plates and observed for a change in color from yellow to red. Disks which did not reveal this color change within 15 min at room temperature were incubated for 1 h at 37°C as previously described (4). Control strains included *Staphylococcus aureus* ATCC 29213, *B. fragilis* ATCC 25285 (positive); *Haemophilus influenzae* ATCC 10211, *Enterococcus faecalis* ATCC 29212, and a laboratory strain of *B. oralis*, HMC 1223 (negative).

Susceptibility testing. Testing of antimicrobial susceptibility to amoxicillin, amoxicillin-clavulanate, ticarcillin, ticarcillin-clavulanate, clavulanate (Beecham Pharmaceuticals, Bristol, Tenn.), cefoxitin, imipenem (Merck Sharpe and Dohme Laboratories, West Point, Pa.), and metronidazole (Searle Pharmaceuticals, Chicago, Ill.) was performed by the agar dilution method recommended by the National Committee for Clinical Laboratory Standards with Wilkins-Chalgren agar containing 5% sterile defibrinated sheep blood to adequately support the growth of fusobacteria and some of the *Bacteroides* species used in this study (3, 21, 24, 25). Quality control strains were included with each run. Plates contained serial doubling dilutions of antimicrobial agents (amoxicillin and amoxicillin-clavulanate, 0.125 to 256 μg/ml; ticarcillin and ticarcillin-clavulanate 0.5 to 64 μg/ml; cefoxitin, 0.5 to 64 μg/ml; and metronidazole, 0.5 to 16 μg/ml). Clavulanate was added to amoxicillin and ticarcillin at a fixed concentration of 2 μg/ml and was also tested alone to 1 to 32 μg/ml to ensure that MICs were at least fourfold above the concentration used in the combinations (3). Imipenem was tested at a single screening concentration of 2 μg/ml. Susceptibility breakpoints used were 4 μg/ml for amoxicillin and amoxicillin-clavulanate, 2 μg/ml for imipenem, 16 μg/ml for metronidazole, 32 μg/ml for cefoxitin (newly proposed by the National Committee for Clinical Laboratory Standards), and 64 μg/ml for ticarcillin and ticarcillin-clavulanate (3).

RESULTS

A total of 64.7% of *Bacteroides* species and 41.1% of fusobacteria were β-lactamase positive (Table 1). Except for *B. denticola* and *B. gracilis*, all *Bacteroides* species contained β-lactamase-positive strains, ranging from 12.5 to 85.0%. Positivity rates were highest for *B. bivius* (85.0%), *B. splanchnicus* (83.3%), *B. eggerthii* (77.8%), and *B. oralis* (77.1%); 54.5% of all black-pigmented *Bacteroides* were β-lactamase positive. Among the fusobacteria, *F. mortiferum* exhibited a high positivity rate (76.9%), with rates for other species ranging between 21.4 and 50.0%; overall, 41.1% of fusobacteria were positive.

The results of susceptibility testing are shown in Tables 2 through 4. All isolates were susceptible to ticarcillin-clavulanate, imipenem, and metronidazole. With the new National Committee for Clinical Laboratory Standards cefoxitin breakpoint of 32 μg/ml, all isolates were also susceptible to cefoxitin. For 10 isolates (2 *B. bivius*, 1 *B. capillosus*, 1 *B. eggerthii*, 1 *B. oralis*, 1 *B. asaccharolyticus*, 1 *B. ureolyticus*, 1 *F. mortiferum*, and 2 *F. varium*) the cefoxitin MIC was 32 μg/ml; 9 of these 10 isolates were β-lactamase positive.

With the addition of clavulanate to amoxicillin and ticar-

TABLE 2. Percent susceptibility of *Bacteroides* species and fusobacteria

Antimicrobial agent (susceptibility breakpoint, μg/ml)	% of the following susceptible (*n*)[a]:					
	Bacteroides spp. (320)		*Fusobacterium* spp. (129)		All organisms (449)	
	+ (207)	− (113)	+ (53)	− (76)	+ (260)	− (189)
Amoxicillin (4)	41.3	99.1	64.2	97.4	45.9	98.4
Amoxicillin-clavulanate (4)	90.8	100.0[b]	88.7	100.0[b]	90.4	100.0[b]
Ticarcillin (64)	92.3	100.0	98.1	100.0	93.4	100.0
Ticarcillin-clavulanate (64)	100.0	100.0[b]	100.0	100.0[b]	100.0	100.0[b]
Cefoxitin (32)	100.0	100.0	100.0	100.0	100.0	100.0
Imipenem (2)	100.0	100.0	100.0	100.0	100.0	100.0
Metronidazole (16)	100.0	100.0	100.0	100.0	100.0	100.0

[a] +, β-Lactamase positive; −, β-lactamase negative.
[b] Many β-lactamase-negative strains were inhibited by clavulanate alone at <16 μg/ml; therefore, results reflect the intrinsic activity of clavulanate as a β-lactam and not as a β-lactamase inhibitor.

TABLE 3. MIC_{50} and MIC_{90}s for β-lactamase-positive species

Organism (*n*)	MIC_{50}/MIC_{90} (μg/ml)						
	Amoxicillin	Amoxicillin-clavulanate	Ticarcillin	Ticarcillin-clavulanate	Cefoxitin	Clavulanate	Metronidazole
Bacteroides spp. (207)	8/256	≤0.12/4	4/64	≤0.5/2	2/16	8/32	1/4
B. melaninogenicus (20)	32/128	≤0.12/1	32/64	≤0.5/2	8/16	16/32	2/4
B. intermedius (14)	16/>256	≤0.12/2	8/128	≤0.5/2	2/8	8/16	1/2
B. oralis (27)	32/256	0.25/8	16/128	≤0.5/2	8/16	16/32	2/4
B. bivius (85)	8/128	≤0.12/8	2/32	≤0.5/1	≤0.5/8	8/32	1/2
B. disiens (15)	8/16	≤0.12/0.5	4/16	≤0.5/1	≤0.5/2	8/8	≤0.5/2
B. oris, *B. buccae*, *B. buccalis*, or *B. loeschei* (12)	8/128	≤0.12/2	4/128	≤0.5/4	2/8	8/32	1/4
B. capillosus (13)	8/64	0.25/2	4/32	≤0.5/1	4/16	8/32	2/4
B. eggerthii (7)	128/256	0.25/128	64/128	1/32	4/32	8/32	2/4
Other (14)[a]	0.5/16	≤0.12/2	4/32	≤0.5/4	1/8	8/32	2/2
Fusobacterium spp. (53)	2/32	0.5/8	2/16	1/8	2/16	16/>32	1/4
F. mortiferum (20)	8/32	0.5/8	8/16	1/8	2/16	32/>32	1/4
F. varium (17)	1/32	0.25/2	2/8	≤0.5/2	2/16	16/>32	1/4
Other (16)[b]	1/8	≤0.12/2	2/16	≤0.5/2	1/8	16/>32	≤0.5/8
All isolates (260)	8/128	≤0.12/4	4/64	≤0.5/2	2/16	8/32	1/4

[a] *B. splanchnicus* (*n* = 5), *B. ureolyticus* (*n* = 6), *B. gingivalis* (*n* = 2), and *B. asaccharolyticus* (*n* = 1).
[b] *F. nucleatum* (*n* = 9), *F. necrophorum* (*n* = 5), and *F. gonidiaformans* (*n* = 2).

cillin, MICs for 90% of strains (MIC_{90}s) of amoxicillin (256 μg/ml) and ticarcillin (64 μg/ml) against β-lactamase-positive *Bacteroides* species decreased to 4 and 2 μg/ml, respectively; for β-lactamase-positive fusobacteria, MIC_{90}s decreased from 32 μg/ml for amoxicillin and 16 μg/ml for ticarcillin to 8 μg/ml for both agents (Table 3). MIC_{90}s for all β-lactamase-positive strains decreased from 128 and 64 μg/ml to 4 and 2 μg/ml for amoxicillin and ticarcillin, respectively. The addition of clavulanate raised amoxicillin susceptibility rates for β-lactamase-positive *Bacteroides* species and fusobacteria from 41.3 to 90.8% and from 64.2 to 88.7%, respectively (Table 2); ticarcillin susceptibility rates for the two organism groups rose from 92.3 to 100% and from 98.1 to 100%, respectively. For all strains, amoxicillin and ticarcillin susceptibility rates rose from 45.9 to 90.4% and from 93.4 to 100%, respectively. Twenty-five β-lactamase-positive isolates were resistant to amoxicillin-clavulanate; MICs (amoxicillin component) were 8 to 32 μg/ml. These isolates (number) were *B. bivius* (nine), *B. capillosus* (one), *B. eggerthii* (one), *B. oralis* (four), *B. gingivalis* (one), *B. melaninogenicus* (two), *B. intermedius* (one), *F. mortiferum* (five), and *F. varium* (one). For 20 of these isolates, however, susceptibility was enhanced with the addition of clavulanate, with amoxicillin MICs being reduced from 64 to >256 to 8 to 32 μg/ml. Clavulanate did not enhance the activity of amoxicillin against the remaining five strains (four *F. mortiferum* and one *B. gingivalis*), for which the amoxicillin MIC was 16 μg/ml. The activity of amoxicillin against 24 other β-lactamase-positive strains (2 *B. splanchnicus*, 2 *B. loeschei*, 3 *B. bivius*, 2 *B. ureolyticus*, 2 *F. nucleatum*, 2 *F. necrophorum*, 3 *F. mortiferum*, 7 *F. varium*, and 1 *F. gonidiaformans*) for which amoxicillin MICs were in the susceptibility range was not enhanced by clavulanate. However, for these strains the amoxicillin MIC_{90} was 16 μg/ml, with 82.7% susceptible, and the ticarcillin MIC_{90} was 8 μg/ml, with 100% susceptible.

Among the β-lactamase-negative strains, three (one each of *B. melaninogenicus*, *F. mortiferum*, and *F. varium*) were resistant to amoxicillin, with MICs of 8 to 16 μg/ml; MICs were not affected by the addition of clavulanate. MIC_{90}s of amoxicillin and ticarcillin for all β-lactamase-negative strains were 1 and 4 μg/ml, respectively (Table 4), with very little variation between species. MIC_{50}s and MIC_{90}s were in the susceptibility range for all combinations (Table 2). Interestingly, many of the strains were susceptible to clavulanate alone at <16 μg/ml; this phenomenon was not seen with β-lactamase-positive strains. The MIC_{50} of clavulanate for β-lactamase-negative strains was 2 μg/ml; that for β-lactamase-positive strains was 8 μg/ml (Tables 3 and 4). However, MIC_{90}s were 32 μg/ml for both groups, limiting the potential clinical value of clavulanate alone.

DISCUSSION

We have recently documented the superiority of β-lactamase testing by the nitrocefin disk method over a microtiter

TABLE 4. MIC_{50}s and MIC_{90}s for β-lactamase-negative species

Organism (*n*)	MIC_{50}/MIC_{90} (μg/ml)						
	Amoxicillin	Amoxicillin-clavulanate	Ticarcillin	Ticarcillin-clavulanate	Cefoxitin	Clavulanate	Metronidazole
Bacteroides spp. (113)	≤0.12/0.5	≤0.12/≤0.12[a]	≤0.5/2	≤0.5/1[a]	≤0.5/4	2/16	1/4
Fusobacterium spp. (76)	≤0.12/0.5	≤0.12/≤0.12[a]	≤0.5/2	≤0.5/≤0.5[a]	≤0.5/8	2/32	1/4
All isolates (189)	≤0.12/1	≤0.12/≤0.12	≤0.5/4	≤0.5/≤0.5	≤0.5/8	2/32	1/4

[a] Many β-lactamase-negative strains were inhibited by clavulanate alone at <16 μg/ml; therefore, results reflect the intrinsic activity of clavulanate as a β-lactam and not as a β-lactamase inhibitor.

nitrocefin assay and therefore chose the former method for the current study (4). We observed significant β-lactamase positivity rates in non-*B. fragilis Bacteroides* species (64.7%) as well as in fusobacteria (41.1%). Previous workers have reported similar high rates of β-lactamase positivity in non-*B. fragilis Bacteroides* species. Rosenblatt (24) documented a 59% positivity rate in strains of *B. melaninogenicus* and other non-*B. fragilis Bacteroides* species, while Bourgault and co-workers (5) found a positivity rate of 52% in these strains. Goldstein and Citron (12) reported 30% of non-*B. fragilis Bacteroides* species to be β-lactamase producers, and Brown (7) reported a 63% positivity rate in this group. As in the current study, all of the above-described studies reported MICs of penicillins against β-lactamase-positive strains to be significantly higher than those against β-lactamase-negative strains.

The high rate of β-lactamase positivity (41.1%) in the 129 fusobacteria tested in the current study contrasts with other North American reports. Studies in the 1970s and early 1980s found fusobacteria highly susceptible to penicillin, with MIC_{90}s of <1 μg/ml, and only 1 resistant strain out of 72 in three studies (8, 10, 20). Later studies showed an increase in the frequency of detection of β-lactamase-producing fusobacteria. In 1983, Aldridge et al. reported 1 of 19 fusobacteria to be β-lactamase positive (1), and in 1986 Bourgault et al. reported 2 positive strains out of 7 (5). Other recent studies did not find any penicillin-resistant fusobacteria in 31 strains studied (7, 16, 23). Tunér et al. described a β-lactamase in *F. nucleatum* in 1985 (31), and Nord et al. reported 4 of 40 fusobacteria to be β-lactamase producers in 1987 (22). Wexler and Finegold reported that for 7 of 38 fusobacteria ampicillin MICs were 32 to >256 μg/ml in 1987 (β-lactamase results not given) (32). The isolation of β-lactamase-producing fusobacteria therefore appears to be increasing, and its clinical significance needs to be assessed.

In the current study, clavulanate was found to greatly enhance the activities of amoxicillin and ticarcillin. Whereas only 45.9% of β-lactamase-producing strains were susceptible to amoxicillin alone, 90.4% were susceptible to amoxicillin-clavulanate, the amoxicillin MIC_{90} being reduced from 128 to 4 μg/ml. With ticarcillin, 90.4% were susceptible to the agent alone, and 100% were susceptible to ticarcillin-clavulanate, the ticarcillin MIC_{90} being reduced from 64 to 2 μg/ml. Our findings confirm previous reports of susceptibility to these agents (3, 9, 12, 18, 19, 23, 30). The effect of clavulanate was not as marked against fusobacteria as against *Bacteroides* species, because of the relatively low β-lactam MICs observed even for β-lactamase-positive strains and the higher β-lactam MICs observed for *F. mortiferum* (Table 3).

The lack of enhancement of the activity of amoxicillin by clavulanate in 10 strains of *Bacteroides* species and 19 strains of fusobacteria (all β-lactamase producers) may not be clinically important, as 24 of these 29 strains (82.7%) had β-lactam MICs in the susceptibility range. Jacobs and coworkers described a new β-lactamase from *B. fragilis* homology group II which is not inhibited by clavulanate (M. R. Jacobs, J. R. Little, S. C. Aronoff, D. M. Shlaes, and P. C. Appelbaum, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 499, 1988). It remains to be seen whether β-lactamases from other *Bacteroides* species and fusobacteria which are not rendered more susceptible to the activity of amoxicillin by clavulanate differ from those of strains which are.

Although organisms of the *B. fragilis* group are clearly resistant to amoxicillin in vitro, with only 14% being susceptible (3), 45.9% of β-lactamase-producing non-*B. fragilis Bacteroides* species and fusobacteria were susceptible to amoxicillin. The clinical significance of this lower level of β-lactam resistance is unclear, although limited clinical and animal data show that β-lactamase production correlates with clinical failure of penicillin therapy (12, 13). The demonstration of β-lactamase production in non-*B. fragilis Bacteroides* species and fusobacteria therefore does not have the same significance that it does in the *B. fragilis* group, and an MIC determination is required to determine the level of resistance; however, β-lactamase-negative strains were ≥98% susceptible to the β-lactams used in this study and did not require the addition of clavulanate. In addition, β-lactamase results are usually available before the identities of isolates have been determined and can guide therapy in a more timely fashion. A negative β-lactamase result may therefore be of more value than a positive one.

The results of the current study emphasize significant rates of β-lactamase production in non-*B. fragilis Bacteroides* species and fusobacteria and indicate the need for β-lactamase testing of these isolates. The high rate of positivity in fusobacteria is noteworthy, and investigation of the nature of the β-lactamases involved is in progress. On the basis of our findings, amoxicillin-clavulanate, ticarcillin, cefoxitin, imipenem, and metronidazole should be suitable agents for the treatment of anaerobic infections with these strains. The addition of clavulanate to ticarcillin does not significantly improve the activity of ticarcillin against these strains, although the MICs are lowered.

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