ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1992, p. 1140–1143
0066-4804/92/051140-04$02.00/0
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Vol. 36, No. 5

# Susceptibilities of 540 Anaerobic Gram-Negative Bacilli to Amoxicillin, Amoxicillin-BRL 42715, Amoxicillin-Clavulanate, Temafloxacin, and Clindamycin

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Received 18 October 1991/Accepted 6 March 1992

**Agar dilution MIC testing of amoxicillin, amoxicillin-BRL 42715, amoxicillin-clavulanate, temafloxacin, and clindamycin against 496 β-lactamase-producing anaerobic gram-negative rods revealed MICs for 90% of the strains tested of 256.0 (amoxicillin), 2.0 (amoxicillin-BRL 42715 and amoxicillin-clavulanate), and 4.0 (temafloxacin and clindamycin) μg/ml. Amoxicillin, temafloxacin, and clindamycin inhibited all 44 β-lactamase-negative strains (MICs for 90% of the strains tested, ≤2.0 μg/ml). BRL 42715 will not be developed, but temafloxacin merits clinical evaluation.**

β-Lactamase production and concomitant resistance to many β-lactams are usually encountered in the *Bacteroides fragilis* group but have been increasingly reported in other anaerobic gram-negative rods (2, 6, 7, 13, 14). We have examined the susceptibilities of 304 *B. fragilis* strains; 171 non-*B. fragilis* group *Bacteroides*, *Prevotella*, and *Porphyromonas* species (142 were β-lactamase positive); and 65 *Fusobacterium* species (50 were β-lactamase positive) to amoxicillin, amoxicillin-BRL 42715 (a potent new β-lactamase inhibitor [8, 9, 17, 23]), amoxicillin-clavulanate, temafloxacin (10, 11, 16, 19), and clindamycin.

Organisms were selected from clinical isolates collected over the past 4 years from diverse sources as previously described (2–6, 13, 14). Prior to being tested, strains were identified (1, 12, 20) as described previously. β-Lactamase production was tested by the nitrocefin disk method (BBL Microbiology Systems, Cockeysville, Md.) (4). MICs of amoxicillin, amoxicillin-clavulanate, amoxicillin-BRL 42715 (SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, United Kingdom), temafloxacin (Abbott Laboratories, Chicago, Ill.), and clindamycin (The Upjohn Co., Kalamazoo, Mich.) were determined by agar dilution (2) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (18). Inhibitors were tested at a fixed concentration of 2 μg/ml. Susceptibilities to amoxicillin (with or without inhibitors), clindamycin, and temafloxacin were evaluated at breakpoints of 2.0, 4.0, and 8.0 μg/ml, respectively (Table 1). When available, NCCLS breakpoints were included in the analyses (18).

β-Lactamase extraction and 50% inhibitory concentration determination of clavulanate, BRL 42715, sulbactam (Pfizer Laboratories, New York, N.Y.), and tazobactam (Lederle Laboratories, Pearl River, N.Y.) were done as described previously (3), with test enzymes from six *B. fragilis* group, one *B. capillosus*, and three *Prevotella* sp. isolates. All extracts were standardized to contain similar units of activity against nitrocefin.

In Tables 1 and 2, percent susceptibility rates of amoxicillin with and without inhibitors are presented at breakpoints of 2.0, 4.0, and 8.0 μg/ml, respectively. Susceptibility data for β-lactamase-producing strains are shown in Table 1. Against all β-lactamase-positive strains, addition of BRL 42715 to amoxicillin lowered the MICs for 50 and 90% of the strains tested ($MIC_{50}$ and $MIC_{90}$, respectively) from 32 and 256 to 0.25 and 2 μg/ml, respectively, and 99% of the strains were susceptible at all three amoxicillin breakpoints (27% of the strains were susceptible to amoxicillin at the NCCLS breakpoint of 4 μg/ml). Addition of clavulanate to amoxicillin lowered the $MIC_{50}$ and $MIC_{90}$ to 0.5 and 2.0 μg/ml, respectively, with 97% of the strains susceptible at the NCCLS amoxicillin breakpoint of 8 μg/ml (18). Temafloxacin and clindamycin showed good activity against β-lactamase-producing strains (95 and 98%, respectively, were susceptible at ≤4 μg/ml), but clustering near the breakpoint was seen with both compounds.

All β-lactamase-negative strains were susceptible to amoxicillin, temafloxacin, and clindamycin (Table 2), and many were susceptible to the inhibitors alone (MICs, <8 μg/ml). Of note were the significantly lower $MIC_{50}$s and $MIC_{90}$s of both temafloxacin and clindamycin against β-lactamase-negative strains (0.25 and 2.0 μg/ml and ≤0.125 and 0.5 μg/ml, respectively) than against β-lactamase-positive strains (Table 2).

Of the 496 β-lactamase-producing strains, the activity of amoxicillin was enhanced by both BRL 42715 and clavulanate in 460 strains; 13 strains showed enhancement only with BRL 42715, and 23 strains showed no enhancement with either inhibitor. Amoxicillin MICs of the latter two groups were lower than those seen in strains showing enhancement ($MIC_{90}$, 4.0 versus 256 μg/ml) (2, 5, 6, 13, 14). All 471 β-lactamase-producing strains which were susceptible to amoxicillin-clavulanate ($MIC_{90}$, 2 μg/ml) were also susceptible to amoxicillin-BRL 42715 ($MIC_{90}$, 1 μg/ml). However, 25 strains resistant to amoxicillin-clavulanate

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TABLE 1. Susceptibility of β-lactamase-positive anaerobes to amoxicillin (alone and combined with BRL 42715 or clavulanate), temafloxacin, and clindamycin

| Organisms (no. of strains) | Antimicrobial agent | MIC (μg/ml) | | | % Susceptible[a] |
|---|---|---|---|---|---|
| | | Range | 50%[b] | 90%[b] | |
| *Bacteroides fragilis* group (304) | Amoxicillin | 0.25–>256 | 32 | >256 | 6, 11, 16 |
| | Amoxicillin-BRL 42715[c] | ≤0.12–>32 | 0.25 | 2 | 99, 99, 99 |
| | Amoxicillin-clavulanate[c] | ≤0.12–>32 | 0.5 | 2 | 91, 94, 96 |
| | Temafloxacin | ≤0.12–>8 | 2 | 4 | 76, 97, 99 |
| | Clindamycin | ≤0.12–>8 | 0.5 | 4 | 86, 98, 100 |
| | BRL 47215 | 8–>32 | 16 | 32 | —[d] |
| | Clavulanate | 8–>32 | 16 | >32 | — |
| Non-*Bacteroides fragilis* group[e] (142) | Amoxicillin | 0.25–>256 | 8 | 128 | 30, 42, 55 |
| | Amoxicillin-BRL 42715 | ≤0.12–4 | ≤0.12 | 1 | 99, 100, 100 |
| | Amoxicillin-clavulanate | ≤0.12–>32 | ≤0.12 | 2 | 92, 97, 99 |
| | Temafloxacin | ≤0.12–8 | 1 | 4 | 73, 94, 100 |
| | Clindamycin | ≤0.12–8 | ≤0.12 | 1 | 92, 98, 100 |
| | BRL 47215 | 8–>32 | 8 | 32 | — |
| | Clavulanate | 8–>32 | 8 | >32 | — |
| *Fusobacterium* species (50) | Amoxicillin | 0.25–128 | 1 | 32 | 72, 84, 84 |
| | Amoxicillin-BRL 42715 | ≤0.12–4 | ≤0.12 | 0.5 | 94, 100, 100 |
| | Amoxicillin-clavulanate | ≤0.12–32 | 0.25 | 2 | 92, 96, 98 |
| | Temafloxacin | 0.25–8 | 2 | 8 | 74, 86, 100 |
| | Clindamycin | ≤0.12–8 | 0.5 | 4 | 84, 94, 100 |
| | BRL 47215 | 8–>32 | 8 | >32 | — |
| | Clavulanate | 8–>32 | 32 | >32 | — |
| All β-lactamase-positive isolates (496) | Amoxicillin | 0.25–>256 | 32 | 256 | 20, 27, 34 |
| | Amoxicillin-BRL 42715 | ≤0.12–>32 | 0.25 | 2 | 99, 99, 99 |
| | Amoxicillin-clavulanate | ≤0.12–>32 | 0.5 | 2 | 91, 95, 97 |
| | Temafloxacin | ≤0.12–>8 | 2 | 4 | 75, 95, 100 |
| | Clindamycin | ≤0.12–8 | 0.25 | 4 | 87, 98, 100 |
| | BRL 47215 | 8–>32 | 8 | 32 | — |
| | Clavulanate | 8–>32 | 16 | >32 | — |

[a] Percentages susceptible at 2, 4, and 8 μg/ml are shown.
[b] MIC inhibiting 50% or 90% of strains.
[c] β-Lactamase inhibitors were tested at a fixed concentration of 2 μg/ml.
[d] —, not applicable.
[e] Includes non-*B. fragilis* group *Bacteroides* species and *Prevotella* and *Porphyromonas* species.

($MIC_{50}$, 16 μg/ml; $MIC_{90}$, >32 μg/ml) were much more susceptible to amoxicillin-BRL 42715 ($MIC_{50}$, 2 μg/ml; $MIC_{90}$, 16 μg/ml). Fifty percent inhibitory concentration ranges were 0.00018 to 1.5 (clavulanate), 0.052 to 3.0 (sulbactam), 0.0024 to 1.8 (tazobactam), and 0.00008 to 0.18 (BRL 42715) μM.

The amoxicillin-clavulanate breakpoints of 8 and 4 μg/ml recommended by the NCCLS (18) are predicated upon a fixed ratio of both compounds and not the fixed-concentration method employed in the current study. However, we feel that previous studies indicate that (i) more clinical relevance may be gained by utilization of the same β-lactam breakpoint with or without an inhibitor and (ii) an amoxicillin breakpoint (with or without an inhibitor) of 4 μg/ml discriminates more accurately between susceptible and resistant anaerobic gram-negative bacilli than does one of 8 μg/ml (2, 5, 6, 13, 14). The rationale for the choice of breakpoint for temafloxacin is based upon the discussion in NCCLS standard M11, which states that maximal dosing of a parenteral antibiotic and the aerobic "intermediate" interpretation are consistent with an interpretation of "susceptible" for anaerobes (18).

The current study shows that BRL 42715, in addition to reported activity against many β-lactamases from aerobes, is also active against β-lactamases of anaerobes. Clindamycin was active against 98% of the strains tested. Because of generalized hospital use of this compound in the United States, clindamycin resistance may occur (22) and should be monitored for. Temafloxacin was active against 95% of all strains at ≤4 μg/ml, but MICs clustered around the breakpoint. However, with a mean peak level in serum of 7.1 μg/ml and a mean trough level in serum of 3.4 μg/ml (10, 19), results point to possible clinical efficacy. Temafloxacin MICs were similar to those described previously (11, 16), i.e., lower than those for ciprofloxacin and ofloxacin (16, 21) and comparable to those for tosufloxacin (6) and sparfloxacin (21).

MICs of clindamycin and temafloxacin against β-lactamase-negative anaerobic gram-negative bacilli were lower than those against β-lactamase-positive strains. This phenomenon has been reported for tosufloxacin, tetracycline, and trospectomycin and may reflect a generalized permeability barrier in β-lactamase-producing strains (6, 15).

In summary, BRL 42715 showed potent inhibitory activity against β-lactamases from anaerobic gram-negative bacilli. Unfortunately, the compound is not being developed. Clindamycin retains its efficacy against gram-negative anaerobes, while clinical studies are required to determine the efficacy of temafloxacin against infections with these organisms.

TABLE 2. Susceptibility of β-lactamase-negative anaerobes to amoxicillin (alone and combined with BRL 42715 or clavulanate), temafloxacin, and clindamycin

| Organisms (no. of strains) | Antimicrobial agent | MIC (μg/ml) | | | % Susceptible[a] |
|---|---|---|---|---|---|
| | | Range | 50%[b] | 90%[b] | |
| Non-*Bacteroides fragilis* group[c] (29) | Amoxicillin | ≤0.12–0.25 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Amoxicillin-BRL 42715[d] | ≤0.12–0.25 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Amoxicillin-clavulanate[d] | ≤0.12 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Temafloxacin | ≤0.12–2 | 0.25 | 2 | 100, 100, 100 |
| | Clindamycin | ≤0.12–2 | ≤0.12 | 0.5 | 100, 100, 100 |
| | BRL 47215 | ≤4–>32 | ≤4 | 8 | —[e] |
| | Clavulanate | ≤4–>32 | ≤4 | 32 | — |
| *Fusobacterium* species (15) | Amoxicillin | ≤0.12–0.25 | ≤0.12 | 0.25 | 100, 100, 100 |
| | Amoxicillin-BRL 42715 | ≤0.12–0.25 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Amoxicillin-clavulanate | ≤0.12–0.25 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Temafloxacin | ≤0.12–4 | 0.25 | 2 | 93, 100, 100 |
| | Clindamycin | ≤0.12–1 | ≤0.12 | 0.25 | 100, 100, 100 |
| | BRL 47215 | 4–32 | ≤4 | 8 | — |
| | Clavulanate | 4–16 | 8 | 16 | — |
| All β-lactamase-negative isolates (44) | Amoxicillin | ≤0.12–0.25 | ≤0.12 | 0.25 | 100, 100, 100 |
| | Amoxicillin-BRL 42715 | ≤0.12–0.25 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Amoxicillin-clavulanate | ≤0.12–0.25 | ≤0.12 | ≤0.12 | 100, 100, 100 |
| | Temafloxacin | ≤0.12–4 | 0.25 | 2 | 98, 100, 100 |
| | Clindamycin | ≤0.12–2 | ≤0.12 | 0.5 | 100, 100, 100 |
| | BRL 47215 | 4–>32 | ≤4 | 8 | — |
| | Clavulanate | 4–>32 | 8 | 16 | — |

[a] Percentages susceptible at 2, 4, and 8 μg/ml are shown.
[b] MIC inhibiting 50% or 90% of strains.
[c] Includes non-*B. fragilis* group *Bacteroides* species and *Prevotella* and *Porphyromonas* species.
[d] β-Lactamase inhibitors were tested at a fixed concentration of 2 μg/ml.
[e] —, not applicable.

Financial assistance from SmithKline Beecham Pharmaceuticals and Abbott Laboratories is gratefully acknowledged.

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