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# Identification and Antimicrobial Susceptibility of 250 *Bacteroides fragilis* Subspecies Tested by Broth Microdilution Methods

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Broth dilution antimicrobial susceptibility tests and biochemical identification of *Bacteroides fragilis* subspecies were performed by micromethods, yielding results within 48 to 72 h of isolation. The subspecies had similar minimal inhibitory concentration end points.

Quantitative antimicrobial susceptibility testing of anaerobic bacteria has been limited to a few research microbiology laboratories (1, 2, 4, 6-8, 11, 14). Even in large medical centers, only periodic anaerobe susceptibility tests may be performed for resistance monitoring and epidemiology (7, 11). In such studies minimum inhibitory concentrations (MICs) are rarely shown separately for the various subspecies of *Bacteroides fragilis* (2). This paper presents simple, cost-effective microdilution methods for susceptibility testing and the identification of *B. fragilis* subspecies.

A total of 250 consecutive strains of anaerobic gram-negative bacilli from surgical cultures were studied. All isolates were presumptively identified as *B. fragilis* by a three-test protocol (growth in 20% bile, esculin hydrolysis, and resistance to a 2-U penicillin G disk). The presumptive method was confirmed by gas-liquid chromatography. Biochemical tests were performed with plastic trays (Canalco-Ames), utilizing 100 μl of substrate and 50 μl of inoculum broth. All biochemical bases (peptone yeast) were prepared without indicator dyes (5). Dispensed biochemical substrates in the trays were reduced in anaerobic jars (3 to 5 h) before inoculation. Positive and uninoculated, negative pH controls were tested in parallel with unknown organisms. pH end points were determined after 48 h of incubation at 35 C (GasPak), using bromocresol purple pH paper and/or a pH electrode. The criteria used for subspecies identification were those of Holdeman and Moore (5) and Sutter et al. (13). Fifteen biochemical tests were utilized, including 12 carbohydrate acid tests and tests for nitrate, indole, and urease.

Microdilution broth susceptibility testing was performed on all of the isolates by techniques similar to those described by Rotilie et al. (9). Brain heart infusion broth containing 0.1 μg of menadione and 0.01 μg of hemin per ml was dispensed in plastic trays containing 80 wells (Micro-Media Systems, Inc., Campbell, Calif.). Nine antimicrobial agents were tested in 7 to 14 dilutions each. The trays were stored at −20 C; for use, they were brought to room temperature and placed in an anaerobic jar for 3 to 5 h before inoculation. The bacterial inoculum was prepared in a concentration of $10^7$ organisms per ml and then automatically dispensed into the tray wells in amounts yielding a final concentration $5 \times 10^5$ colony-forming units per ml. MICs, or the lowest concentrations inhibiting visible growth, were determined at 48 h. Most of the strains exhibited readable end points at 18 to 24 h (9, 10). Quality-control organisms required known MICs were tested in parallel (*B. fragilis subsp. thetaiotaomicron* and *S. faecalis* ATCC 29212).

Of the 250 *B. fragilis* strains, *B. fragilis* subsp. *fragilis* was tested most often. The prevalence of other subspecies was: *B. fragilis* subsp. *thetaiotaomicron* > *B. fragilis* subsp. *distasonis* > *B. fragilis* subsp. *vulgatus* > *B. fragilis* subsp. other. No *B. fragilis* subsp. *ovatus* strains were isolated.

Resistance to the penicillins (Table 1) was in the order: *B. fragilis* subsp. *fragilis* > *B. fragilis* subsp. *thetaiotaomicron* > *B. fragilis* subsp. other = *B. fragilis* subsp. *vulgatus* > *B. fragilis* subsp. *distasonis*. The penicillin and carbenicillin MICs for all isolates were ≥4 μg/ml. All isolates were inhibited by 8 μg of chloramphenicol per ml (Table 2). The mean MICs of clindamycin for *B. fragilis* subsp. *thetaiotaomicron* isolates was 0.5 to 1.0 μg/ml. Only 34% were inhibited by 0.5 μg/ml as compared with 75 to 97% for other subspecies. However,

TABLE 1. *In vitro susceptibility of B. fragilis subspecies to penicillin G and carbenicillin*

| Subspecies (no.) | Antimicrobic[a] | Cumulative % susceptible at an MIC (μg/ml) of: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | >512 |
| *B. fragilis* subsp. *fragilis* (169) | Pen | 3 | 14 | 47 | 79 | 93 | 95 | 97 | 99 | 100 |
| | Carb | | 12 | 33 | 56 | 82 | 95 | 98 | 99 | 100 |
| *B. fragilis* subsp. *thetaiotaomicron* (44) | Pen | 2 | 30 | 73 | 95 | | 98 | | 100 | |
| | Carb | | 18 | 41 | 91 | 98 | | 100 | | |
| *B. fragilis* subsp. *distasonis* (23) | Pen | 22 | 65 | 87 | 100 | | | | | |
| | Carb | | 43 | 100 | | | | | | |
| *B. fragilis* subsp. *vulgatus* (8) | Pen | 38 | 50 | 75 | 100 | | | | | |
| | Carb | | 38 | 63 | 75 | 100 | | | | |
| *B. fragilis* subsp. other (6) | Pen | | 50 | 83 | | 100 | | | | |
| | Carb | | 50 | | 83 | 100 | | | | |

[a] Pen, Penicillin G; Carb, carbenicillin.

TABLE 2. *In vitro susceptibility of B. fragilis subspecies to chloramphenicol, clindamycin, and tetracycline*

| Subspecies (no.) | Antibiotic[a] | Cumulative % susceptible at an MIC (μg/ml) of: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.25 | 0.5 | 1.0 | 2 | 4 | 8 | 16 | 32 | >32 |
| *B. fragilis* subsp. *fragilis* (169) | Chlo | | | 2 | 7 | 67 | 99 | 100 | | |
| | Clin | 85 | 97 | 98 | 99 | | | 100 | | |
| | Tet | 26 | 40 | 47 | 48 | 52 | 69 | 94 | | 100 |
| *B. fragilis* subsp. *thetaiotomicron* (44) | Chlo | | | | 2 | 73 | 100 | | | |
| | Clin | 20 | 34 | 73 | 95 | 100 | | | | |
| | Tet | 22 | 41 | 45 | 50 | 59 | 70 | 82 | | 100 |
| *B. fragilis* subsp. *distasonis* (23) | Chlo | | 4 | | 30 | 83 | 100 | | | |
| | Clin | 35 | 83 | 96 | 100 | | | | | |
| | Tet | 52 | 65 | | | | 87 | 100 | | |
| *B. fragilis* subsp. *vulgatus* (8) | Chlo | | | | 13 | 87 | 100 | | | |
| | Clin | 63 | 75 | 88 | 100 | | | | | |
| | Tet | 63 | 75 | | | 88 | 100 | | | |
| *B. fragilis* subsp. other | Chlo | | | | 17 | 100 | | | | |
| | Clin | 67 | 83 | 100 | | | | | | |
| | Tet | 33 | 50 | | | | | 83 | | 100 |

[a] Chlo, Chloramphenicol; Clin, clindamycin; Tet, tetracyline.

TABLE 3. *Selection of single concentrations for conduct of antimicrobial tests to assist bacteroides identification*

| MIC (μg/ml) | No. of isolates | | | |
|---|---|---|---|---|
| | Colistin | Gentamicin | Rifampin | Vancomycin |
| >16 | 237 | 250 | 0 | 207 |
| 16 | 9 | 0 | 0 | 37 |
| 8 | 1 | 0 | 0 | 6 |
| 4 | 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 |
| 0.5 | 1 | 0 | 8 | 0 |
| ≤0.25 | 0 | 0 | 242 | 0 |

all isolates were inhibited by a clinically achievable clindamycin concentration, 4 μg/ml. A bimodal tetracycline MIC distribution was demonstrated. The subspecies tetracycline resistance order was: *B. fragilis* subsp. *fragilis* = *B. fragilis* subsp. *thetaiotaomicron* = *B. fragilis* subsp. other (approximately 50% at a break point of 4 μg/ml) > *B. fragilis* subsp. *distasonis* = *B. fragilis* subsp. *vulgatus*.

Four additional MIC tests similar to the disk method of Finegold et al. (3) and Sutter et al. (12, 13) were utilized for genus level identification. A common pattern of colistin, gentamicin, and vancomycin resistance and rifampin sensitivity was noted (Table 3). A *Bacteroides* genus level identification confidence value of 99.6% was achieved for these strains, with the following single concentrations: colistin and gentamicin, 16 μg/ml; vancomycin, 8 μg/ml; and rifampin, 2 μg/ml.

The data presented here outline simple, inexpensive, broth micromethods for *B. fragilis* antibiotic susceptibility testing and subspecies identification. MICs and 15 biochemical tests

were performed, and results were obtained within 48 to 72 h of initial isolation. There was minimal variation among the *B. fragilis* subspecies in their antibiotic susceptibility. Only the mean MIC of clindamycin was higher for *B. fragilis* subsp. *thetaiotaomicron* than for the other subspecies.

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