Infections due to anaerobic bacteria can be severe and life-threatening. Susceptibility testing of anaerobes is not frequently performed in laboratories, but such testing is important to direct appropriate therapy. Anaerobic resistance is increasing globally, and resistance trends vary by geographic region. An overview of a variety of susceptibility testing methods for anaerobes is provided, and the advantages and disadvantages of each method are reviewed. Specific clinical situations warranting anaerobic susceptibility testing are discussed.

Keywords. anaerobe; anaerobic bacteria; resistance; susceptibility; susceptibility test methods.

Anaerobic infections can be severe and life-threatening, and their increasing incidence is due in part to the high numbers of patients with complex underlying diseases [1]. Studies demonstrate that failure to direct appropriate anaerobic therapy leads to poor clinical response [2]. Anaerobic collection, transport, and manipulation of culture isolates can be time-consuming and difficult to perform correctly. Empirical broad-spectrum antimicrobial therapy for anaerobic infections has often been instituted long before results of susceptibility testing are available.

However, antimicrobial bacterial resistance among anaerobic bacteria is increasing globally, as demonstrated by numerous surveys in Europe, the United States, Canada, and New Zealand [3–6]. Differences in resistance patterns may be due to the variety of susceptibility testing methodology used, the selective antibiotic pressures associated with antimicrobial usage, and the lack of uniformity in adoption of interpretive breakpoints.

This article covers typical resistance patterns and recent changes in resistance of certain organisms over time. Anaerobic susceptibility testing methods and their challenges are reviewed.

RESISTANCE PATTERNS OF ANAEROBIC BACTERIA

Bacteroides fragilis Group

The members of the B. fragilis group are among some of the least susceptible anaerobes to antibiotics. Resistance of Bacteroides species is linked to outcome, even in the presence of mixed infections [4, 7]. The B. fragilis group consists of 24 species including Bacteroides fragilis, Bacteroides vulgatus, Bacteroides ovatus, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides caccae, and Parabacteroides distasonis.

Fewer than 3% of Bacteroides isolates are susceptible to penicillin and ampicillin [8]. Penicillin and cephalosporin resistance is mediated by the cepA and cfxA genes. The chromosomal cepA gene is a cephalosporinase encoding for resistance to cephalosporins and aminopenicillins but not piperacillin or β-lactam–β-lactamase inhibitor combinations (BLBLIs). The cfxA gene encodes for high-level resistance to cefoxitin and other β-lactams [9]. Although β-lactamases are the principal mechanism of resistance to penicillins, the expression of altered penicillin-binding proteins can also
lead to resistance [10]. Membrane modifications, such as porin losses, may further increase the minimum inhibitory concentrations (MICs) to β-lactams and BLBLIs. Currently, ticarcillin-clavulanate and piperacillin-tazobactam are active against >90% of the *B. fragilis* group [3, 11, 12]. Carbapenems are generally active against the *B. fragilis* group, and most US-based studies report susceptibility rates of 98.5%–99% [4, 13, 14]. Carbapenem resistance is usually mediated by a chromosomal zinc metallo-β-lactamase enzyme encoded by the *cflA* gene [15]. Although cefepime were used in the past for anaerobic coverage, the Infectious Diseases Society of America (IDSA) recently stated that cefotetan and clindamycin are no longer recommended as therapy for community-acquired intra-abdominal infections in adults due to increasing resistance among the *B. fragilis* group [16]. Clindamycin resistance is mediated by *erm* genes located on transferable plasmids that can also carry tetracycline resistance genes [17]. Worldwide, clindamycin resistance is increasing and approaches 60% [3, 4, 12, 18]. Although rare, metronidazole-resistant strains of *B. fragilis* have been reported and are associated with the *nim* gene [3, 11, 15]. However, the presence of this gene does not invariably lead to resistance, and MICs must be obtained. Of 206 *B. fragilis* isolates in one study, the *nim* gene was detected in 7.3%, but metronidazole MICs ranged from 1.5 mg/L (susceptible) to >256 mg/L (resistant) [19]. Susceptibility rates to moxifloxacin are highly variable across studies, and isolates are acquiring resistance rapidly. Moxifloxacin susceptibility rates range from 30% to 60% for the *B. fragilis* group, and such resistance is mediated by *gyrA* mutations, efflux pumps, or topoisomerase gene modifications [12]. Tigecycline MICs are relatively low in surveillance studies, but there have been reports of resistance [6]. Susceptibility within the *B. fragilis* group varies by species, with *B. fragilis* being the most susceptible. *Parabacteroides distasonis* demonstrates high MICs to β-lactams, and *B. thetaiotaomicron* also demonstrates higher resistance rates to various antimicrobials in comparison to other members of the *B. fragilis* group. A survey of 6574 *B. fragilis* group isolates collected from 10 US medical centers confirmed that susceptibility rates differed widely by species [4]. In this series, *B. ovatus* was more resistant to the carbapenems; *B. vulgatus* was more resistant to piperacillin-tazobactam and showed the highest resistance rates to moxifloxacin (54%); *P. distasonis* was more resistant to ampicillin-sulbactam and cefoxitin; and *B. ovatus* and *B. uniformis* showed higher resistance rates to moxifloxacin (39% and 41%, respectively). However, a 2008 practice survey of anaerobes across the United States reported that 33% of reference laboratories did not identify *B. fragilis* group isolates to the species level [20]. Susceptibility rates among *B. fragilis* group are decreasing worldwide. Snydman et al reported a decrease in susceptibility of approximately 25% over 11 years to ampicillin-sulbactam in *P. distasonis* and *B. vulgatus* [4].

### Prevotella, Porphyromonas, and Other Anaerobic Gram-Negative Rods

*Porphyromonas* species are generally susceptible to β-lactams, clindamycin, and metronidazole [21]. One-fourth to one-third of *Porphyromonas* species produce β-lactamases, and clindamycin resistance has been observed in a minority of strains [22]. Compared with *Porphyromonas*, approximately 95% of *Prevotella* species is resistant to penicillin and ampicillin [22]. A recent Belgian study showed that clindamycin susceptibility in *Prevotella* has been decreasing from 91% in 1993–1994 to 69% in 2012 [23]. Susceptibility to moxifloxacin, metronidazole, carbapenems, and amoxicillin-clavulanate is typically ≥90%. Penicillin resistance in *Fusobacterium* species ranges from 4% to 15% and is generally due to the production of β-lactamases [22]. *Fusobacterium varium*, *Fusobacterium mortiferum*, and *Fusobacterium nucleatum* are most often reported to produce β-lactamases, and >90% of *Fusobacterium necrophorum* are susceptible to cephalosporins and cephamycins [14, 24]. *Fusobacterium* species are typically susceptible to metronidazole, BLBLIs, cephaparins, carbapenems, and clindamycin.

### Gram-Positive, Non-Spore-Forming Rods

*Actinomyces*, *Propionibacterium*, *Bifidobacterium*, and the *Eubacterium* group are usually susceptible to β-lactams and BLBLIs [25, 26]. *Propionibacterium*, *Actinomyces*, *Bifidobacterium*, and *Lactobacillus* are usually resistant to metronidazole. Clindamycin shows moderate activity against these bacteria [27]. Decreasing clindamycin susceptibility of *Propionibacterium acnes* is associated with prior topical acne therapy [28]. Vancomycin shows some activity against certain species of *Lactobacillus*; however, MICs >256 µg/mL are common for the most frequently isolated lactobacilli from human specimens, *Lactobacillus casei* and *Lactobacillus rhamnosus* [29]. Telavancin has shown good activity against some *Lactobacillus* species [30]. Newer antimicrobial agents such as linezolid, daptomycin, and dalbavancin exhibit excellent in vitro activity against most anaerobic gram-positive species [31, 32].

### Gram-Positive, Spore-Forming Rods

Of the clostridial species, *Clostridium perfringens* is one of the most susceptible to penicillin, but clindamycin resistance is increasing, including up to 14% of blood isolates of *C. perfringens* in one study [5, 26, 33]. Tetracycline resistance (defined as MIC >2 µg/mL) has been documented in up to 75% of *C. perfringens* isolates obtained from commercial poultry, but there are few data in humans [34, 35]. The activities of tetracycline against other clostridial species and of doxycycline against clostridia in general have not been well documented [35]. Drugs that maintain activity against non-*perfringens* *Clostridium* species include piperacillin, BLBLIs, carbapenems, metronidazole, and vancomycin [36].

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Antimicrobial agents lacking significant activity include ampicillin, aminoglycosides, trimethoprim-sulfamethoxazole, and clindamycin. Resistance to clindamycin is typical for many species, including *Clostridium tertium*, *Clostridium difficile*, and the “RIC” group of clostridia—namely, *Clostridium innocuum*, *Clostridium innocuum*, and *Clostridium clistodiforme*. In a study of 540 clinical isolates of *Clostridium* species, tigecycline was highly active, with an MIC of 0.25 µg/mL [37]. *Clostridium difficile* is usually susceptible to metronidazole and vancomycin but resistant to β-lactams, fluoroquinolones, and clindamycin [38]. Outbreaks of fluoroquinolone-resistant *C. difficile* strains have been associated with increased mortality [39]. Strains resistant to rifampin or rifaximin associated with *rpoB* mutations have also been linked to clinical failure [38]. Metronidazole-resistant strains are rare but have been documented [40]. A recent surveillance study of *C. difficile* isolates collected from 14 European countries reported that more than one-half of isolates were multidrug resistant, with the majority resistant to moxi-floxacin, clindamycin, erythromycin, and rifampicin [41]. Fidaxomicin shows excellent activity against *C. difficile*, but reduced susceptibility due to drug target modifications has been documented in vitro [42].

**ANAEROBIC SUSCEPTIBILITY TESTING**

Susceptibility testing is performed to obtain information on the predicted response of the bacteria to antibiotics in the form of an MIC, which is defined as the lowest concentration of the antibiotic that inhibits growth of organisms. Drugs are typically tested at 2-fold doubling (log2) serial dilutions (ie, 2 µg/mL, 4 µg/mL, 8 µg/mL, etc). MICs may be obtained by utilizing the Clinical and Laboratory Standards Institute (CLSI)—defined agar or broth microdilution methods or by using a variety of commercially available methods. Commercial methods include Etest strips (bioMérieux, Durham, North Carolina), M.I.C.Evaluator strips (M.I.C.E.; Thermo Fisher Scientific, Basingstoke, United Kingdom), and Sensititre panels (Trek Diagnostics Systems, Thermo Fisher Scientific, Cleveland, Ohio). Etests and M.I.C.Evaluator strips are gradient diffusion methods. Agar and broth microdilution methods are usually employed by research or reference laboratories. Advantages and disadvantages of anaerobic antimicrobial susceptibility testing (AST) methods are listed in Table 1.

**Table 1. Advantages and Disadvantages of Antimicrobial Susceptibility Testing Methods for Anaerobic Bacteria**

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar dilution</td>
<td>• Reference method against which other methods are compared</td>
<td>• Labor-intensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Expertise required for performance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Considerable time is involved in setting up testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Not amenable/appropriate for testing small numbers of organisms</td>
</tr>
<tr>
<td>Broth microdilution</td>
<td>• Multiple antimicrobials (≥10) can be tested per isolate</td>
<td>• Currently recommended by CLSI only for <em>Bacteroides fragilis</em> group organisms</td>
</tr>
<tr>
<td></td>
<td>• Commercial panels are available</td>
<td>• Shelf life of frozen panels may be limited</td>
</tr>
<tr>
<td></td>
<td>• Laboratories can customize their own panel of antibiotics to test</td>
<td>• Poor growth by some strains has been shown</td>
</tr>
<tr>
<td>MIC gradient diffusion method</td>
<td>• Ease of use</td>
<td>• Expensive for surveillance purposes</td>
</tr>
<tr>
<td></td>
<td>• Precise MIC value is obtained</td>
<td>• Metronidazole resistance can be overestimated if anaerobiosis is inadequate</td>
</tr>
<tr>
<td></td>
<td>• Convenient for testing of a few individual isolates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Multiple drugs can be tested at one time</td>
<td></td>
</tr>
<tr>
<td>Rapid β-lactamase disk testing</td>
<td>• Rapid (at most 30 min to results)</td>
<td>• Only tests for β-lactamases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be used on a limited number of bacterial species</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Negative result must be followed up with an MIC test for accurate prediction of β-lactam susceptibility</td>
</tr>
</tbody>
</table>

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration.
In a 2008 practice survey of anaerobic culture and AST in US hospitals, Goldstein and colleagues reported that only 21% (21/98) of hospital laboratories performed anaerobic AST in-house (as opposed to outsourcing to a reference laboratory) [20]. Twenty hospital laboratories reported sending their isolates out for susceptibility testing, and the remaining 60% (58/98) of laboratories did not offer any anaerobic AST. In 1990, 70% of hospital laboratories performed anaerobic AST, but only 33% of hospital laboratories did so in 1993. This decline may be explained by the fact that the disk diffusion and broth disk elution methods, which were the predominant methods of susceptibility testing in 1993, are no longer considered appropriate for susceptibility testing. In the 2008 survey, 65% of hospital laboratories that performed anaerobic AST used Etest methodology [20]. The remaining of hospital laboratories used microbroth dilution, and none used agar dilution.

Procedural guidelines for anaerobic AST have been published by CLSI, but the US Food and Drug Administration and European Committee on Antimicrobial Susceptibility Testing also develop breakpoints [45–47]. Occasionally, there are differences in interpretive criteria between organizations, which may be explained by differences in dosages, administration intervals, inoculum size, or test media. It is important for clinicians to be aware of which breakpoints are being used. Antimicrobial susceptibility testing in anaerobes relies primarily on phenotypic characterization, although a few molecular assays exist (eg, nim gene for metronidazole resistance and cfiA for carbapenem resistance) [48]. As molecular data on anaerobic susceptibility are gathered, it is possible that additional molecular assays could become available.

**Agar Dilution**

The CLSI agar dilution procedure is the gold standard reference method for anaerobic AST [45]. Different concentrations of antimicrobial agents are prepared in 2-fold dilutions, added to molten agar, poured into Petri dishes, and allowed to solidify. A standardized inoculum of bacterial cells is spotted onto the surface of each plate with an inoculator, replicator, or pipette. After 48 hours of incubation, the plates are examined visually for the lowest concentration of antibiotic that inhibits growth. Agar dilution testing requires considerable time, labor, and expertise and is not intended for testing of single isolates. Thus, it is not a technique that many clinical laboratories employ.

**Broth Microdilution**

The broth microdilution method is less labor-intensive than agar dilution. In this assay, drugs in serial 2-fold dilutions are added to small volumes of broth liquid media pipetted in wells of plastic microdilution trays or plates. A standard inoculum of bacterial cells is added into each well. MICs are read after 48 hours of incubation. Multiple antibiotics (10 or more) can be tested at one time, and the laboratory can design its own drug panels. However, this technique is limited by poor growth of certain oxygen-sensitive anaerobes, and is currently validated by CLSI for use only with *B. fragilis* group [46].

### MIC Gradient Diffusion Method

The Etest and M.I.C.E. strips are thin plastic strips that are impregnated on one face with an increasing gradient concentration of an antimicrobial agent (Figure 1). The other face of the strip is marked with an MIC scale with increasing antibiotic concentrations, including increments between doubling dilutions (ie, 1 µg/mL, 1.5 µg/mL, 1.75 µg/mL, 2 µg/mL, etc). A Brucella blood agar plate supplemented with hemin and vitamin K₁ is prereduced in an anaerobic environment to rid the media of toxic oxygen metabolites. A standard inoculum of bacterial cells is swabbed onto the plate. The Etest or M.I.C.E. strip is placed antibiotic face down. The MIC is read at the intersection of the lower part of the ellipse with the corresponding number on the test strip. Results are generally available at 48 hours; however, clostridial species and *B. fragilis* group isolates usually grow rapidly enough to be read at 24 hours. MIC gradient diffusion methods are easier to use in routine clinical laboratory practice than agar dilution. A significant advantage of the MIC gradient diffusion method is the ability to generate a quantitative and precise MIC. Additionally, several drugs can be tested at one time, and laboratories can tailor testing to their needs. However, Etest strips cost approximately US$2–$3 apiece. Etest results generally correlate well with agar dilution, but the MIC gradient diffusion method can overestimate

![Figure 1. Penicillin Etest (bioMérieux) of *Bacteroides fragilis* on Brucella blood agar plate supplemented with hemin and vitamin K₁ (Anaerobe Systems). The minimum inhibitory concentration of this isolate equals 32 µg/mL.](https://academic.oup.com/cid/article-abstract/59/5/698/2895712)
metronidazole resistance if anaerobiosis is not adequate [49]. Because the activity of metronidazole is dependent upon an active intermediate that requires the reduced atmosphere of anaerobiosis, it is important to utilize anaerobic control organisms when setting up such testing.

Commercially Available Broth Microdilution Panels

Two commercially available panels for anaerobic susceptibility testing are available. The Anaerobe Sensititre panel (ANO2, Thermo Fisher) is a panel of antimicrobials of varying 2-fold dilutions (Figure 2) [50]. A second anaerobic susceptibility panel is available through Oxoid (Thermo Fisher) [51].

Rapid β-Lactamase Disk Testing

This disk test evaluates for the presence of β-lactamase enzyme. Bacterial colonies are smeared onto a disk embedded with a chromogenic cephalosporin. If the organism expresses β-lactamase, the disk will change colors after reagent is added. Although most reactions occur within 5–10 minutes, some β-lactamase-positive strains may react more slowly (up to 30 minutes). If the disk is positive, the organism is considered to be resistant to penicillin, ampicillin, and amoxicillin. Because β-lactamases are not the sole mediator of resistance in anaerobes, a negative test does not rule out penicillin or ampicillin resistance resulting from alterations of penicillin-binding proteins [52]. Therefore, a negative result by the rapid β-lactamase disk test must be followed by an MIC test for accurate prediction of β-lactam susceptibility. Also, the β-lactamase disk testing should not be performed on members of the B. fragilis group, as they are presumed to be resistant due to almost universal presence of β-lactamases.

Disk Diffusion

Disk diffusion (Kirby-Bauer) tests should not be performed for anaerobic bacteria for the purpose of obtaining susceptibility results, as the results are inaccurate and do not correlate with the agar dilution method [46]. Special-potency antibiotic disks of vancomycin (5 µg), kanamycin (1000 µg), and colistin (10 µg) are occasionally used in the laboratory as an aid in preliminary identification of some anaerobes based on patterns of resistance [53]. However, due to the high antibiotic concentrations in these disks, these disks are not intended for use in determining antibiotic susceptibility for therapeutic purposes.

STRATEGIES FOR TESTING AND REPORTING OF SUSCEPTIBILITY DATA

Antimicrobial Testing Guidelines

For individual patient management, anaerobic AST should be performed when (1) selection of an active agent is critical for disease management, (2) long-term therapy is being considered, (3) anaerobes are recovered from sterile body sites, or (4) the

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**Table 2. Indications for Susceptibility Testing of Anaerobic Bacteria**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistence of infection despite adequate therapy with an appropriate therapeutic regimen</td>
<td>Any anaerobe</td>
</tr>
<tr>
<td>Particular species of bacteria is implicated in disease</td>
<td>Bacteroides fragilis, Prevotella species, Clostridium species other than C. perfringens, Bilophila wadsworthia, Fusobacterium species, Sutterella wadsworthensis</td>
</tr>
<tr>
<td>Long-term therapy needed</td>
<td>Anaerobic organisms involved in osteomyelitis, endocarditis, or brain abscess</td>
</tr>
<tr>
<td>Pivotal role of antimicrobial agent in clinical outcome</td>
<td>B. fragilis group implicated in osteomyelitis or joint infection</td>
</tr>
<tr>
<td>Infections of specific body sites</td>
<td>Brain abscess, Endocarditis, Prosthetic devices or graft infections, Bacteremia</td>
</tr>
</tbody>
</table>

*The above suggestions are examples only and are not intended to be used as all-inclusive lists.*
infection persists despite adequate therapy with an appropriate therapeutic regimen (Table 2). In cases of a single recovered anaerobic pathogen from culture, AST should be performed. CLSI suggests certain antimicrobial agents to be considered for testing and reporting on anaerobic organisms (Table 3) [46]. For gram-negative anaerobes, penicillin or ampicillin may be tested and/or reported selectively for all except the B. fragilis group, which are almost uniformly resistant. For non-spore-forming gram-positive anaerobes, resistance to metronidazole is common, and ampicillin and penicillin are recommended for primary testing. Although CLSI MIC breakpoints for amoxicillin have not been established for anaerobes, amoxicillin breakpoints are considered equivalent to ampicillin [46]. The remaining supplemental antimicrobials are helpful to test when strains are resistant to primary drugs or for use in patients who are allergic to primary drugs.

**Antibiograms**

Establishment of an antibiogram outlining patterns of resistance on a periodic basis is helpful in guiding antimicrobial therapy. The CLSI suggests gathering susceptibility data from at least 30 isolates of the same genus or species collected over the period of a year to obtain a reasonable number of isolates upon which to estimate susceptibilities [54]. Examples of cumulative antimicrobial susceptibility reports for B. fragilis group and other anaerobes from across the United States are included in the CLSI documents M100 and M11 [45, 46].

**CONCLUSIONS**

Susceptibility among anaerobes differs from species to species and also from region to region. Surveillance studies have shown that susceptibility trends of anaerobes to certain antimicrobial agents are changing, with more resistance recognized in many parts of the world. Despite the difficulties inherent in anaerobic AST, testing should be performed and observed for trends over time.

**Note**

*Potential conflicts of interest.* Author certifies no potential conflicts of interest.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


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Table 3. Suggested Antimicrobial Agents for Testing and Reporting on Anaerobic Organisms*

<table>
<thead>
<tr>
<th>Bacteroides fragilis Group and Other Gram-Negative Anaerobes</th>
<th>Gram-Positive Anaerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Penicillin</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>Amoxicillin-clavulanate</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>Ampicillin-sulbactam</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>Piperacillin-tazobactam</td>
</tr>
<tr>
<td>Ticarcillin-clavulanate</td>
<td>Ticarcillin-clavulanate</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>Doripenem</td>
<td>Doripenem</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>Ertapenem</td>
</tr>
<tr>
<td>Imipenem</td>
<td>Imipenem</td>
</tr>
<tr>
<td>Meropenem</td>
<td>Meropenem</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Metronidazole</td>
</tr>
</tbody>
</table>

*Only 1 of the antimicrobial agents in each box needs to be tested under usual circumstances, as antibiotics that are listed together demonstrate similar clinical efficacy and interpretive results.

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