# Antimicrobial Resistance in Anaerobes

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**The development of antibiotic resistance in anaerobic bacteria has a tremendous impact on the selection of antimicrobial agents for empirical therapy. Susceptibility studies have documented the emergence of antimicrobial resistance and indicate distinct differences in resistance patterns related to individual hospitals, geographic regions, and antibiotic-prescribing regimens. Resistance to β-lactam drugs, clindamycin, tetracyclines, and 5-nitroimidazoles (metronidazole) has been observed. The prime mechanism for resistance to β-lactam agents is the production of β-lactamases. Resistance to clindamycin is mediated by modification of the ribosome. Tetracycline resistance is mediated by both tetracycline efflux and ribosomal protection. 5-Nitroimidazole resistance appears to be caused by a combination of decreased antibiotic uptake and decreased nitroreductase activity. The level of chloramphenicol susceptibility remains quite high, whereas uniform resistance to aminoglycosides and quinolones is observed. Understanding the mechanisms of resistance is critical for both informed selection of antimicrobial therapy and the design of new antimicrobial agents.**

Anaerobic bacteria are an important class of human and animal pathogens. Less is often known about anaerobic bacteria than most aerobic or facultative bacteria, but their participation in human health and well-being should not be undervalued. Although clinical laboratories may not always test for these organisms, their susceptibility patterns vary among the different groups, both with respect to organism and with respect to different classes of antimicrobial agents. Thus, it is important to appreciate the variations in resistances and to relate these variations to mechanisms of resistance.

Of the anaerobic bacteria, *Bacteroides* species are among the most important clinically for two reasons. First, they are the microorganisms most often isolated from patients with suppurative anaerobic infections. Second, they are the anaerobic bacteria with the broadest spectrum of recognized resistances to antimicrobial agents. As such, *Bacteroides* species have been instrumental in the study of resistance and its transfer among anaerobic bacteria.

Consequently, more is known about antimicrobial resistance in *Bacteroides* than in any other anaerobic bacteria. For these reasons, the major emphasis of this article will be on the mechanisms of antibiotic resistance in *Bacteroides,* as an update to our recent review on the mechanisms of resistance in *Bacteroides* [1].

## Susceptibility Patterns Among Anaerobes

Susceptibility studies are conducted in many countries as an attempt to monitor the development of resistance in specific organisms. A summary of data from worldwide studies that identified the appearance of resistance or an increase in frequency of resistance to different classes of antimicrobial agents is provided in table 1. The agents included were β-lactam drugs, clindamycin, metronidazole, and chloramphenicol, antimicrobials that have been used extensively to treat anaerobic infections.

Penicillins, including the ureidopenicillin piperacillin, are β-lactam agents that members of the *Bacteroides fragilis* group are resistant to. However, the addition of a β-lactamase inhibitor often allows a labile penicillin to regain its original activity. Cefoxitin, a cephalosporin formerly highly active against anaerobes, is exhibiting decreased potency in many of the recent surveys (table 1). Imipenem remains the most potent β-lactam agent. It is notable that Australia is the one country in which MICs of the β-lactam agents are consistently lower than those in the United States for non-*fragilis Bacteroides* isolates.

Clindamycin and metronidazole are both quite active against the *B. fragilis* group, although a frequency of resistance to clindamycin of up to 25% has been reported in localized areas such as southern Europe and Japan [6, 12, 15]. Susceptibility patterns for other anaerobes including fusobacteria, clostridia, and propionibacteria are quite different as the penicillins have retained potent activity. A few strains of *Clostridium perfringens* have been reported to be metronidazole-resistant [15]. It is also notable that resistance to metronidazole is seen in propionibacteria, while other agents are quite active.

## Mechanisms of Antibiotic Resistance

### β-Lactam Agents

As indicated above, the β-lactam agents most active against anaerobic bacteria are imipenem and combinations of a β-lactam agent with a β-lactamase inhibitor. When organisms are resistant to penicillins, the addition of a β-lactamase inhibitor is often

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**Table 1.** Summary of data from worldwide studies on the susceptibility patterns for anaerobes.

| Organism(s), country (survey year[s]) | $MIC_{90}$ (μg/mL) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pen | Amp | Amp/Sulb* | Tic/CA | Pip | Pip/Taz† | Cfox | Imi | Cm | Mtz | Chl | [Reference] |
| *Bacteroides fragilis* group | | | | | | | | | | | | |
| Australia (1991) | 128 | 128 | 8.0 | 4.0 | 128 | ND | 32 | 0.50 | 8.0 | 1.0 | 8.0 | [2] |
| Canada (1989–1990) | >128 | ND | ND | 4.0 | >128 | 32 | 64 | 0.50 | 4.0 | 0.50 | 8.0 | [3] |
| Canada (1990–1991) | ND | 64 | 8.0 | 4.0 | 32 | ND | 32 | 0.50 | 4.0 | 2.0 | ND | [4] |
| Indonesia (1988–1990) | ND | ND | ND | ND | ND | ND | 32 | ND | 4.0 | ≤1.0 | 4.0 | [5] |
| Spain (1989) | ND | ND | 4.0 | 4.0 | 128 | ND | 32 | 0.50 | >256 | 1.0 | 4.0 | [6] |
| United States (1988) | >64 | >64 | 8.0 | 16 | >128 | ND | >32 | 1.0 | 2.0 | 1.0 | ND | [7] |
| United States (1989) | ND | >128 | 8.0 | 16 | >128 | ND | 16 | 1.0 | 4.0 | 2.0 | 4.0 | [8] |
| United States (1987–1991) | ND | ND | 4.0 | ND | 128 | 16 | 32 | 0.25 | 8.0 | 2.0 | ND | [9] |
| *B. fragilis* | | | | | | | | | | | | |
| Australia (1991) | 128 | 128 | 4.0 | 0.25 | 32 | ND | 16 | 0.25 | 1.0 | 1.0 | 8.0 | [2] |
| Canada (1989–1990) | >128 | ND | 4.0 | 1.0 | 128 | 4.0 | 32 | 0.25 | 2.0 | 0.50 | 4.0 | [3] |
| Europe (1988–1989) | ND | 64 | ND | ND | ND | ND | 16 | 0.25 | 0.50 | 1.0 | 4.0 | [10] |
| Italy (ND) | 64 | 128 | ND | ND | 64 | ND | 16 | 1.0 | 1.0 | 0.50 | ND | [11] |
| Japan (1986–1989) | ND | 200 | ND | ND | 100 | ND | 50 | 0.78 | >200 | 0.78 | ND | [12] |
| Japan (1986–1991) | ND | 200 | ND | ND | 100 | ND | 25 | 0.78 | 200 | 0.78 | ND | [13] |
| United States (1988) | >64 | >64 | 8.0 | 2.0 | 128 | ND | 32 | 1.0 | 2.0 | 2.0 | ND | [7] |
| United States (ND) | ND | ND | 4.0 | ND | >128 | ND | 32 | 0.50 | 2.0 | 1.0 | ND | [14] |
| *Bacteroides*, all other species | | | | | | | | | | | | |
| Australia (1991) | 8.0 | 32 | 2.0 | 1.0 | 8.0 | ND | 4.0 | 0.06 | 1.0 | 1.0 | 2.0 | [2] |
| United States (1988) | >64 | >64 | 4.0 | 4.0 | >128 | ND | 32 | 0.50 | 2.0 | 2.0 | ND | [7] |
| *Fusobacterium* species | | | | | | | | | | | | |
| Australia (1991) | 0.25 | 8.0 | 2.0 | ND | ND | ND | 4.0 | 4.0 | 0.12 | 0.25 | 0.12 | [2] |
| United States (1988) | ≤0.13 | 2.0 | 0.5 | ≤0.25 | 1.0 | ND | 4.0 | 0.03 | 0.25 | ≤0.13 | ND | [8] |
| United States (ND) | ND | ND | 2.0 | ND | 0.25 | ND | 2.0 | 0.50 | 0.13 | 0.25 | ND | [14] |
| *Propionibacterium acnes* | | | | | | | | | | | | |
| Australia (1991) | 0.06 | 0.25 | 0.25 | 0.03 | 0.25 | ND | 0.25 | 0.03 | 0.12 | 32 | 1.0 | [2] |
| *Propionibacterium* species | | | | | | | | | | | | |
| United States (ND) | ND | ND | 0.125 | ND | 1.0 | ND | 1.0 | 0.03 | ≤0.06 | >128 | ND | [14] |
| *Clostridium perfringens* | | | | | | | | | | | | |
| Australia (1991) | 0.50 | 2.0 | 1.0 | 16 | 1.0 | ND | 2.0 | 0.06 | 2.0 | 2.0 | 4.0 | [2] |
| United States (ND) | ND | ND | 0.125 | ND | 0.50 | ND | 2.0 | 0.13 | 1.0 | 1.0 | ND | [14] |

NOTE. Amp = ampicillin; CA = clavulanic acid fixed at 2 μg/mL; Cfox = cefoxitin; Chl = chloramphenicol; Cm = clindamycin; Imi = imipenem; Mtz = metronidazole; ND = no data provided; Pen = penicillin G; Pip = piperacillin; Sulb = sulbactam; Taz = tazobactam; Tic = ticarcillin.

* Fixed ratio of 2:1.

† Ratio of 8:1.

effective against these isolates. Cefoxitin has lost its role as a β-lactam agent with guaranteed potency, but it still is used when strains are susceptible after testing. Generally, narrow-spectrum penicillins and cephalosporins should not be considered efficacious agents against *Bacteroides* species, but for some isolates expanded-spectrum cephalosporins will be effective.

Resistance to the β-lactam agents can be caused by any of the three major resistance determinants: an altered killing target with poor binding for the β-lactam agent (e.g., low-affinity penicillin-binding proteins [PBPs]), decreased permeability, and the presence of an inactivating enzyme (i.e., a β-lactamase with the ability to hydrolyze the β-lactam drug to form an inactive entity). The most common mechanism at this time is inactivation by one of the various groups of β-lactamases found in the anaerobic bacteria. A lengthy discussion of resistance to the β-lactam agents has been published recently [1], and only an update of more current information will be included in the following section.

*β-Lactamases.* Production of β-lactamases by anaerobes is a common occurrence; as many as 97%–100% of *Bacteroides* isolates in the United States [16, 17] and 76% of such isolates in Great Britain [18] produce β-lactamases. Sixty-five percent of non-*fragilis Bacteroides* species produce β-lactamases [19]. Most enzymes from *Bacteroides* are chromosomally mediated and are produced constitutively. They include enzymes with serine at the active site as well as enzymes known to be metalloenzymes requiring an active site with $Zn^{++}$ for hydrolysis of the β-lactam agent to occur.

Production of β-lactamases by other anaerobic bacteria has been less well studied, but enzyme production by *Clostridium butyricum* [20, 21], *Clostridium clostridioforme* [22], *Clostridium ramosum* [23], *Prevotella, Porphyromonas* [24], and *Fusobacterium* [19, 24] has been reported. Although only isolated strains of *Clostridium* appear to produce β-lactamases, *Prevotella, Porphyromonas,* and fusobacteria produce these enzymes more frequently; Jacobs et al. [17] and Appelbaum and coworkers [19] reported that 71%, 30%, and 41% of these strains, respectively, were β-lactamase producers. Enzymes produced by clostridia are generally inducible. The exception is the β-lactamase produced by *C. ramosum* that Matthew [23]

**Table 2.** Representative $\beta$-lactamases from anaerobic bacteria.

| Enzyme group,* strain | pI | Relative hydrolysis rate | | | | | | | | $IC_{50}$ ($\mu M$) | | | Class† | [Reference(s)] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pen G | Amp | Clox | Clor | Cthn | Ctax | Cfox | Imi | CA | Sulb | EDTA | | |
| 1 | | | | | | | | | | | | | | |
| *Bacteroides intermedius* | ND | ND | <1 | ND | 100 | 30 | ND | ND | ND | >10 | >10 | ND | ND | [26] |
| 2a | | | | | | | | | | | | | | |
| *Fusobacterium nucleatum* F-21 | 4.8 | 100 | 420 | ND | 4.9 | 0.25 | ND | ND | 0.03 | 2.0 | 1.0 | ND | ND | [27, 28] |
| 2c | | | | | | | | | | | | | | |
| *Clostridium butyricum* NBL3 | 4.4 | 560 | 890 | ND | 100 | 1.7 | 0.2 | ND | 0.01 | ≤0.04 | 3.0 | ND | ND | [28, 29] |
| 2d | | | | | | | | | | | | | | |
| *Bacteroides fragilis* GN11499 | 6.9 | 140 | 500 | 380 | 100 | 80 | ND | <1 | ND | <0.1 | <0.1 | ND | ND | [30] |
| *Clostridium clostridioforme* | 4.2 | 370 | ND | 1,800 | 100 | ND | ND | <4 | ND | 3.6 | 59 | – | ND | [22] |
| 2e | | | | | | | | | | | | | | |
| *Bacteroides bivius* 691 | 5.6 | 2.0 | ND | 2 | 100 | 25 | 40 | ND | ND | 0.08 | 0.1 | >100 | ND | [31] |
| *Bacteroides capillosus* 902 | 4.2 | 11.0 | ND | ND | 100 | 45 | <0.1 | ND | ND | 0.08 | 0.1 | >100 | ND | [31] |
| *Bacteroides disiens* 802 | 4.2 | 2.0 | ND | 2 | 100 | 36 | 35 | ND | ND | 1.8 | 1.9 | >100 | ND | [31] |
| *Bacteroides distasonis* GAI2095 | ND | 17.0 | 22 | 7.8 | 100 | 34 | 104 | ND | ND | ND | ND | ND | ND | [32] |
| *B. fragilis* CS30 | 4.9 | 1.0 | ND | ND | 100 | ND | ND | ND | ND | <1 | ND | ND | A | [33] |
| *B. intermedius* 820 | 4.2 | 12.0 | ND | ND | 100 | 65 | 145 | ND | ND | 0.01 | 0.02 | >100 | ND | [31] |
| *Bacteroides loescheii* 201 | 5.0 | 11.0 | ND | 0 | 100 | 830 | 110 | ND | ND | 0.1 | 0.52 | >100 | ND | [31] |
| *Bacteroides melaninogenicus* 184 | 4.3 | 4.0 | ND | 4 | 100 | 67 | 76 | ND | ND | 0.17 | 0.9 | >100 | ND | [31] |
| *Bacteroides oralis* 182 | 4.3 | 7.0 | ND | 0 | 100 | 55 | 26 | ND | ND | 0.04 | 0.03 | >100 | ND | [31] |
| *Bacteroides uniformis* WAL-7088 | 4.6 | 10 | ND | ND | 100 | ND | ND | ND | ND | <1 | ND | ND | A | [34] |
| *Bacteroides vulgatus* CLA341 | ND | 11 | 7.2 | ND | 100 | 68 | 1.0 | <0.01 | ND | 1.0 | <1 | – | A | [35] |
| [?] | | | | | | | | | | | | | | |
| *B. distasonis* TAL7860 | 6.9 | ND | ND | ND | ND | 100 | 34 | + | 0.6 | 0.29 | 0.64 | >10 | ND | [36] |
| *B. fragilis* R212 | 5.1 | + | ND | ND | + | ND | ND | +‡ | ND | 1.0 | 1.0 | – | ND | [18] |
| [3] | | | | | | | | | | | | | | |
| *B. fragilis* TAL3636 | 5.2 | 460 | 460 | 1,640 | 100 | 68 | 230 | 24 | 460 | >500 | >500 | 240 | B | [37, 38] |
| *B. fragilis* 10-73 | ND | 250 | 180 | ND | 100 | ND | 90 | 8 | 130 | >500 | ND | <100 | ND | [39] |

NOTE. Relative hydrolysis rates are based on a standard of 100. Cephaloridine is the standard for comparison among all agents except for the enzyme group 2a, for which penicillin G is the standard. Amp = ampicillin; CA = clavulanic acid; Cfox = cefoxitin; Clor = cephaloridine; Clox = cloxacillin; Ctax = cefotaxime; Cthn = cephalothin; $IC_{50}$ = inhibitory concentration of 50%; Imi = imipenem; ND = no data provided; Pen G = benzylpenicillin; pI = isoelectric point; Sulb = sulbactam; ? = not enough information for a firm assignment; + = presence of hydrolyzing activity but no quantitative data provided; – = absence of hydrolyzing activity but no quantitative data provided.

* Enzymes classified according to Bush et al. [25].

† Molecular class as defined by Ambler [40]. Only those enzymes for which a sequence has been determined are listed with a class designation.

‡ Inactivation detected microbiologically. Relative $V_{max} < 1$ compared with that of Clor. Cfox inactivation decreased in the presence of CA.

reported; this $\beta$-lactamase was the common plasmid-mediated TEM-1 enzyme.

Functional classifications [25] of the $\beta$-lactamases from anaerobic bacteria are given in table 2. Most enzymes produced by the anaerobes have acidic isoelectric points. Many enzymes from the *B. fragilis* group are group 2e cephalosporinases that can be inhibited by the classical $\beta$-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam), thus explaining the susceptibilities of many *Bacteroides* strains to the combinations of a $\beta$-lactam agent with a $\beta$-lactamase inhibitor. These enzymes account for most $\beta$-lactamases produced by *Bacteroides* strains. Several of these enzymes have now been sequenced and have been confirmed to belong to the molecular class A serine cephalosporinases [33–35], which have smaller molecular sizes than the inducible group 1 (molecular class C) cephalosporinases from gram-negative bacteria. Only one strain of *Bacteroides intermedius* has been reported to produce a group 1 cephalosporinase that is not inhibited by clavulanic acid [26].

An unusual set of cefoxitin-hydrolyzing enzymes from several members of the *B. fragilis* group has been reported [1] (table 2). These enzymes have not been completely characterized but may eventually be assigned to group 2e. Although exact rates of hydrolysis were not determined for the enzymes, HPLC revealed that the group 2e (class A) CfxA $\beta$-lactamase from *Bacteroides vulgatus* degraded cefoxitin slowly in overnight cultures, but hydrolysis could not be detected in standard spectrophotometric assays [35]. Because the $\beta$-lactamase-producing organisms are clinically resistant to cefoxitin, it is possible that slow enzymatic hydrolysis is coupled with decreased permeability.

Fusobacteria and clostridia produce penicillinases rather than cephalosporinases. These organisms produce at least three groups of enzymes, although not all substrates have been tested with all enzymes. Penicillinases from fusobacteria and *C. butyricum* have been reported to be inhibited by clavulanic acid, whereas the penicillinase from *C. clostridioforme* was not inhibited by any of the three commercially available β-lactamase inhibitors [22]. This behavior is characteristic of enzymes belonging to molecular class D β-lactamases, the group 2d cloxacillin-hydrolyzing enzymes.

Production of metallo-β-lactamases is perhaps the most disturbing development over the past 10 years in terms of resistance to β-lactam agents in *B. fragilis.* These enzymes, which require $Zn^{++}$ at the active site, readily hydrolyze all β-lactam agents except the monobactam agents [38], which have no antibacterial activity against *Bacteroides.* They also cannot be inhibited by the available β-lactamase inhibitors. Although these enzymes are usually chromosomally mediated, a plasmid-mediated metallo-β-lactamase has been reported in Japan [39]. The appearance of this set of enzymes has put the clinical use of carbapenems, previously the β-lactam agents most active against these organisms, into jeopardy.

*Permeability.* Decreased permeability has often been associated with increased β-lactamase production as a cause of resistance to antimicrobial agents in gram-negative bacteria. Several studies have indicated that permeability factors can vary among strains of *B. fragilis* [36, 37, 41]. In certain *B. fragilis* strains, resistance was also correlated with both reduced permeability and expression of enzymatic activity [41]. In another study, cefoxitin resistance was correlated with a decrease in outer-membrane permeability and the loss of an outer-membrane protein with a molecular size of 49–50 kD [42].

*PBPs.* Binding to PBPs is the critical factor that determines whether a β-lactam agent will be effective as an antimicrobial agent. PBPs function in the terminal stage of cell wall synthesis and are essential for growth of bacteria. When a β-lactam agent is able to compete successfully for the active site of an essential PBP, cell death will occur. In *Bacteroides* strains, three to five PBPs can exist: a PBP 1 complex with one to three different enzymes, PBP 2, and PBP 3. These PBPs are assumed to correspond to the essential high-molecular-weight PBPs found in aerobic gram-negative bacteria. There may be other low-molecular-weight PBPs, but the number of these proteins varies among strains, thereby suggesting that they are probably not critical for growth [43].

Most β-lactam agents bind well to PBP 2, with good binding to the PBP 1 complex. Monobactam agents such as aztreonam seem to have very poor affinity for the *B. fragilis* PBPs, thus accounting for their lack of antimicrobial activity against these organisms [44]. Although resistance to cephalosporins can be attributed to β-lactamase production in most *Bacteroides* strains, decreased affinity for PBP 3 was also demonstrated in *B. fragilis* G-232 from Japan [45]. Cefoxitin resistance in selected *Bacteroides* strains has also been attributed to decreased binding to PBP 2 or the PBP 1 complex [42, 46].

**Clindamycin**

In general, the rate of clindamycin resistance in *Bacteroides* species continues to remain relatively low [2–12, 47], and this drug remains effective as treatment of infections due to *Bacteroides.* However, the rate of resistance varies dramatically between different institutions and countries. Resistance rates can range from almost 0 to >20% [2–12, 47]. Therefore, surveillance of resistance is essential in assessing the utility of clindamycin as a therapeutic agent at a given facility.

The three classic mechanisms of antibiotic resistance (inactivation of the drug, altered permeability, and altered target site [i.e., the ribosome]) have all been reported as mechanisms of clindamycin resistance [48, 49]. Macrolide/lincosamide/streptogramin B (MLS) resistance is a representative of the latter mechanism and is the most common mechanism of clindamycin resistance in *Bacteroides* species. DNA and protein sequence analysis and biochemical evidence indicate that MLS resistance in *Bacteroides* occurs by a mechanism similar to that of clindamycin resistance in staphylococci [48–57].

In staphylococci, resistance is mediated by methylation of the 23S rRNA at one of two adenine residues [50]. This methylation prevents effective binding of clindamycin to the ribosomes and renders them resistant to the drug's inhibitory effects. Supporting this same mechanism of resistance in *Bacteroides* species is the findings of Reig et al. [52] and Jimenez-Diaz et al. [56]; these researchers demonstrated that ribosomes isolated from a *B. vulgatus* strain with clindamycin resistance, which was induced with either clindamycin or erythromycin, showed decreased susceptibility to inactivation by clindamycin compared with ribosomes isolated from a clindamycin-susceptible strain or a strain with clindamycin resistance that was not induced. This observation indicates that resistance to clindamycin is at the level of the ribosome and most likely occurs via methylation of the rRNA, although methylation has not yet been directly demonstrated.

Three different, but very closely related (>95% homologous), MLS resistance genes have been cloned from various *Bacteroides* strains. All three genes lie within a transposon or on a conjugal element: *ermF* is encoded on Tn*4351, ermFS* is encoded on Tn*4551,* and *ermFU* is encoded on a *B. vulgatus* conjugal element [53–55]. In addition to being highly homologous with each other, their encoded proteins have sequence identities that are highly similar to those of the MLS resistance genes from gram-positive organisms [53–55]. Most clindamycin-resistant *Bacteroides* harbor an *erm* gene related to one of the three genes mentioned above. However, not all clindamycin-resistant *Bacteroides* contain DNA sequences that cross-hybridize with the *ermF* gene [57–59], thus indicating that another unrelated MLS resistance gene or another mechanism of resistance is also present in *Bacteroides* species.

Conjugal transfer of clindamycin resistance, first reported in 1979 by researchers at three laboratories [60–62], was shown to be plasmid-mediated. Many of these plasmids are self-transmissible and range in size from 14.6 kilobases (kb) (pBFTM10) to 41 kb (pIP411 and pBF4) [61] to ~82 kb (pBI136) [63]. Studies with pBFTM10 and pBF4 have demonstrated that the clindamycin resistance genes harbored by these plasmids are carried on transposons Tn*4400* and Tn*4351,* respectively [64, 65]. Resistance to clindamycin has also been documented to be chromosomally encoded. In several cases, chromosomal resistance to clindamycin has been linked with resistance to tetracycline; in fact, the clindamycin resistance gene lies within the tetracycline resistance transfer element (see below) [65–67]. Clindamycin resistance can be inducible as well as constitutive.

**Tetracycline**

Resistance to tetracycline is nearly universal in *Bacteroides,* with the rate of resistance exceeding 80%–90% at most institutions [10, 68]. In fact, most surveys of antimicrobial resistance in *Bacteroides* no longer include data on susceptibility to tetracycline. Because of the high rate of resistance, tetracycline is no longer an effective first-line antibiotic for the empirical treatment of bacteroides infections as it was through the 1960s. Tetracyclines can no longer be used as treatment of bacteroides infections without susceptibility testing.

Modification or protection of the target site is the only documented mechanism of tetracycline resistance in *Bacteroides* species. The *tetQ* gene encodes a protein that is able to render the ribosomal protein synthesis machinery resistant to the protein synthesis inhibitory effects of the tetracycline class of antibiotics [59, 69, 70]. Several *Bacteroides tetQ* genes have been cloned, and their DNA sequences have been determined [69, 70]. TetQ is the most distantly related tetracycline resistance protein that functions via ribosomal protection; it is ~40% homologous with TetM and TetO proteins and has been proposed to represent a new class of ribosomal protection proteins [69, 70].

DNA cross-hybridization studies indicate that a *tetQ* or *tetQ*-related gene is present in most tetracycline-resistant *Bacteroides* isolates [59]. However, the identification of tetracycline-resistant isolates that do not contain *tetQ* DNA sequences indicates that another mechanism (such as tetracycline efflux) or another class of ribosomal protection proteins also contributes to tetracycline resistance. In fact, a *tetM*-related determinant has been identified in some tetracycline-resistant isolates of *Bacteroides ureolyticus* [71].

Two tetracycline resistance genes have recently been identified in *C. perfringens.* The *tetA*(P) and *tetB*(P) genes form an operon encoding two unrelated proteins that bestow tetracycline resistance mediated by two different mechanisms [72]. The *tetA*(P) gene product is a tetracycline efflux pump, while *tetB*(P) encodes a protein conferring tetracycline resistance at the level of the ribosome [72]. The TetB(P) protein has sequences that are significantly similar to those of other TetM-like proteins that specify a ribosomal protection mechanism of tetracycline resistance [72].

Two additional genes related to tetracycline resistance have been identified in *Bacteroides* species. The *tetX* gene product is able to inactivate tetracycline by oxidation of the molecule [73, 74]. The TetX protein, however, is active only under aerobic conditions and has not been demonstrated to be functional in *Bacteroides* [73, 75]. A gene encoding a protein that is able to actively produce tetracycline efflux has been identified in *Bacteroides* [76, 77]. However, this efflux pump is not able to bestow tetracycline resistance on *Escherichia coli* and therefore is not believed to contribute to tetracycline resistance in *Bacteroides* [77].

Tetracycline resistance mediated by the *tetQ* gene is both inducible [69, 78] and transferable [60, 79]. This transfer of tetracycline resistance was first reported by Privitera et al. in 1979 [60, 79]. The frequency of transfer of tetracycline resistance is generally very low unless the cells are preexposed to tetracycline [80–82]. The mechanism of transfer is by conjugation mediated by the tetracycline resistance transfer element itself [78, 83, 84]. The regulation of transfer is controlled by a classical prokaryotic two-component regulatory system [78, 84]. The two regulatory genes, *rteA* and *rteB,* are located in the *tetQ* operon downstream from the *tetQ* gene [84]. Their expression is greatly enhanced in the presence of tetracycline, thus explaining the increased transfer following exposure to tetracycline.

The *rteA* gene encodes RteA, the cytoplasmic membrane protein component of the system [84]. The *rteB* gene encodes RteB, the regulatory response protein that may elicit its effect through an interaction with a $\sigma^{54}$-like protein [84]. RteB plays an essential role in the transfer and mobilization of the tetracycline resistance transfer element. A third gene, *rteC,* has also been demonstrated to be involved in self-transfer of tetracycline resistance [78]. Its gene product, RteC, follows RteB in the regulation cascade. The precise role of RteC in the regulation of the transfer process has not been elucidated.

In addition to regulating the movement of the tetracycline resistance transfer element, RteA and RteB are also involved in the regulation of transfer of unlinked chromosomal elements termed nonreplicating *Bacteroides* units (NBUs) [59, 82–85]. Although most NBUs do not carry an identifiable phenotype, a cefoxitin-hydrolyzing $\beta$-lactamase gene (*cfxA* [35]) has been shown to reside on an NBU [86]. Transfer of the cefoxitin-hydrolyzing $\beta$-lactamase is enhanced by pretreatment with tetracycline [86, 87].

These tetracycline resistance transfer elements reside in the chromosome. [59, 80, 81]. The elements are large, with estimated sizes ranging from 70 to 80 kbp [83], and often also harbor other resistance genes (e.g., *ermF*) [66]. Except for their large size, the tetracycline resistance transfer elements in *Bacteroides* are similar to the conjugal transposon Tn*916* in *Enterococcus faecalis* that was described by Franke and Clewell [88].

### 5-Nitroimidazoles (Metronidazole)

Metronidazole, the first 5-nitroimidazole to be used clinically, was introduced in 1960, but it was not until 1978 that Ingham et al. [89] reported the first clinical isolate of *B. fragilis* that was metronidazole-resistant after long-term therapy. Subsequent reports have included descriptions of metronidazole-resistant *Bacteroides* isolates from patients not treated with metronidazole [90–92]. One explanation for these resistant isolates is that metronidazole was inactivated by another bacterium. It has been reported that *E. faecalis* is able to inactivate metronidazole and protect *B. fragilis* from its antimicrobial effects in mixed culture [93]. Nevertheless, rates of metronidazole resistance remain extremely low, generally <1% [2–12, 47], and this drug remains highly effective as treatment of bacteroides infections.

The 5-nitroimidazoles, including metronidazole, tinidazole, and ornidazole, must be reduced to form the active antibacterial agent [94]. This reduction is stable only under anaerobic conditions as it is quickly reversed in the presence of oxygen [94, 95]. Resistance to metronidazole is often associated with reduced nitroreductase activity and decreased uptake of the drug [94, 95]. Both of these conditions occur simultaneously [94, 95]. Because entry of 5-nitroimidazoles into the cell depends on the rate of reduction of the nitro group, a decrease in the reducing environment within the cell will result in reduced nitroreductase activity as well as reduced uptake of the drug.

Decreased pyruvate:ferredoxin oxidoreductase activity in combination with a compensatory increase in the lactate dehydrogenase activity results in a decrease in the reducing state of the cell [94, 95]. The ease with which this change in enzyme activities can be accomplished by the cell is unknown. However, the phenotype in a metronidazole-resistant isolate has been identified [96].

Two genes, *nimA* and *nimB,* that are able to confer moderate- to high-level metronidazole resistance have been identified [97]. Screening of a group of metronidazole-resistant *B. fragilis* isolates indicated that all of the isolates harbored DNA sequences that hybridized with either an *nimA* or *nimB* DNA probe, while none of the metronidazole-susceptible isolates screened contained DNA sequences that cross-hybridized with an *nimA* or *nimB* DNA probe [98]. The DNA sequence of the *nimA* and *nimB* genes is ~73% similar, and presumably they represent two classes of genes that confer resistance via the same mechanism [97]. The mechanism of resistance has not been elucidated, but drug uptake studies indicate that active efflux or reduced drug penetration was not involved [97].

The *nimA* and *nimB* genes have been localized both to the chromosome and to various plasmids [99, 100]. The plasmids harboring the *nimA* or *nimB* gene have not been demonstrated to be self-transmissible, but these plasmids can be mobilized by other conjugal elements or acquired by transformation [101]. It is interesting that the transcriptional start information for both the *nimA* and *nimB* genes that have been sequenced is provided by an insertion sequence (IS) element integrated 12–14 bases upstream from the protein-coding region. For the *nimA* gene, this element is IS*1168* [97]. A very closely related IS element provides the transcriptional start information for the *nimB* gene [97]. IS*1168* is nearly identical to an IS element, IS*1186,* that has been identified to provide transcriptional initiation signals for the *Bacteroides* metallo-$\beta$-lactamase gene [97, 102].

### Chloramphenicol

Most antimicrobial susceptibility surveys have not reported resistance to chloramphenicol [2, 3, 8, 10]. This lack of resistance may be due in part to the infrequent use of chloramphenicol as treatment of bacterial infections. However, failure of chloramphenicol treatment for a patient with meningitis and ventriculitis caused by *B. fragilis* was reported [103].

Two different classes of chloramphenicol resistance genes have been identified in *Bacteroides* species. Both classes of genes confer resistance via drug inactivation, either by nitroreduction at the *p*-nitro group on the benzene ring [104] or by acetylation [105, 106]. In the latter case, the resistance was transferable and was associated with the transfer of a 39.5-kb plasmid, pRYC3373 [106].

### Aminoglycosides

Aminoglycoside resistance is universal in anaerobic bacteria. This common resistance is not the result of decreased sensitivity of the target to the drug, as both streptomycin and gentamicin are able to bind to and inhibit protein synthesis occurring on *B. fragilis* and *C. perfringens* ribosomes in a cell-free system [107]. Drug inactivation also does not account for this resistance, as no drug inactivation has been seen with cell extracts of either *B. fragilis* or *C. perfringens* [107]. Rather, resistance is the result of failure of the drug to reach its target.

Uptake of aminoglycosides is a two-step process involving an energy-independent phase and an energy-dependent phase. An oxygen- or nitrogen-dependent electron transport system provides the energy necessary for the energy-driven phase of drug uptake. Strictly anaerobic bacteria lack this electron transport system and therefore do not import aminoglycosides [108]. Supporting this statement is the observation that aminoglycosides do not accumulate within *B. fragilis* or *C. perfringens* [107]. It can, therefore, be surmised that these compounds are not active against *Bacteroides* and clostridia simply because they do not reach their target site.

### Quinolones

Although highly effective against many aerobic bacteria, the quinolones and fluoroquinolones generally show poor activity against most anaerobic bacteria. MICs of quinolones for most *Bacteroides* isolates are ≥2 μg/mL [109–114]. Different sur-

**Table 3.** Mechanisms of antimicrobial resistance in anaerobic bacteria.

| Antimicrobial agent(s) | Mechanism(s) of resistance |
|---|---|
| β-Lactam agents | Drug inactivation (β-lactamases), decreased permeability (gram-negative organisms), altered target site (penicillin-binding proteins) |
| Clindamycin | Altered target site (methylation of 23S rRNA) |
| Tetracycline | Altered target site (ribosomal protection) |
| 5-Nitroimidazoles | Decreased nitroreductase activity, decreased drug uptake |
| Chloramphenicol | Inactivation of drug (nitroreduction or acetylation) |
| Aminoglycosides | No drug uptake |
| Quinolones | Unknown (decreased permeability and/or uptake) |

veys report MICs of various quinolones that range from 0.8 μg/mL [115] to >400 μg/ml [116, 117]. Studies determining if the low rate of susceptibility is a consequence of poor drug penetrability, low affinity of the drugs for the target, DNA gyrase, or another mechanism have not been performed. However, decreased cell permeability has been suggested to be responsible for the observed resistance to both cefoxitin and norfloxacin in a number of clinical isolates of *Bacteroides* [116].

One reservation to the use of quinolones as treatment of anaerobic infections is the observation that the quinolones are not bactericidal but are bacteriostatic under anaerobic conditions [118, 119]. However, there is the hope that despite a poor history, some of the newer quinolones (such as WIN57273, tosufloxacin, and CI960) may prove to be therapeutically effective as treatment of bacteroides infections [120].

## Discussion

Rates of resistance in the anaerobes, particularly *Bacteroides* species, have not increased dramatically since we last reviewed this subject in 1992 [1–12]. The mechanisms of antimicrobial resistance in aerobic and anaerobic bacteria (table 3) continue to remain the same. However, some features of antimicrobial resistance and expression of the resistance determinants in *Bacteroides* differ from those seen in many other bacterial species.

Many of the resistance determinants identified in *Bacteroides* reside on the chromosome. Plasmids harboring multiple antibiotic resistance genes that readily move from one isolate to another are not a major feature of *Bacteroides.* The tetracycline resistance gene lies on a conjugal element that is most often integrated within the chromosome [80, 83, 84]. With the exception of the metallo-β-lactamase gene in Japan, which can be located on a plasmid [39], this gene is found to be chromosomally encoded in the other strains that have been studied [37, 83]. The *nimA* and *nimB* genes have been found on the chromosome and on plasmids, although not self-mobilizing plasmids [99, 101]. The MLS genes have been identified to reside on transposons and conjugal elements often integrated within the chromosome [55, 66].

The chromosomal location of many of these resistance genes may tend to slow the spread of resistance. However, it should not be overlooked that some of the resistance markers (e.g., the tetracycline resistance *tetQ* gene) are located on self-mobilizing elements whose transfer is enhanced by pretreatment with the very drug for which the markers encode resistance [80, 83]. This phenomenon may in large part be responsible for the rapid dissemination and high incidence of tetracycline resistance in *Bacteroides* species.

It is also interesting that many of the *Bacteroides* resistance genes are transcribed from transcriptional initiation signals provided by an IS element integrated within the promoter rather than from their natural promoters (table 4). This circumstance is the case for the *ermF* and *ermFS* genes (whose transcriptional initiation signals are provided by IS*4351* [53, 54]), the metallo-β-lactamase gene *ccrA* (also known as *cfiA;* at least two different IS elements have been identified that provide the transcriptional initiation signals [102, 121]), and the *nimA* and *nimB* genes [97]. In general, when expression is provided by a promoter encoded on an IS element, the antimicrobial resistance is constitutive not inducible, which tends to be the case for most resistance genes.

This finding leads to the speculation as to why these genes are not transcribed from their natural promoters: were the genes acquired from another bacterium whose transcriptional initiation signals are not optimal for expression in *Bacteroides,* were they acquired without the regulatory genes needed to permit optimal expression, or is the inducer for their expression a compound other than the one for which they impart resistance? The answers to these questions are unknown. It is clear however, that IS element–driven transcriptional gene activation impacts on the spread and rate of antimicrobial resistance in *Bacteroides.*

It has been demonstrated that the metallo-β-lactamase gene is present in >1%–3% of *Bacteroides* isolates, yet only a fraction of these isolates are resistant to imipenem [39, 41, 122, 123]. The metallo-β-lactamase-positive imipenem-susceptible isolates can easily convert to being imipenem-resistant in a

**Table 4.** Insertion sequence elements providing the transcriptional initiation information for different *Bacteroides* resistance genes.

| Resistance gene(s) | Transcriptional promoter(s) | [Reference(s)] |
|---|---|---|
| *ermF* and *ermFS* genes* | IS*4351* | [53, 54] |
| Metallo-β-lactamase gene, *ccrA* (*cfiA*) | IS*942*, IS*1186* | [102, 121] |
| *nimA* and *nimB* genes† | IS*1168* | [97] |

* Macrolide/lincosamide/streptogramin B resistance.
† 5-Nitroimidazole resistance.

single step by the insertion of an IS element into the promoter [102, 122]. The IS element provides the transcriptional initiation information, and expression of the imipenem-hydrolyzing metallo-$\beta$-lactamase becomes constitutive. It is interesting that among *Bacteroides* species, the IS elements that have been identified to provide transcriptional initiation signals to antibiotic resistance genes are found almost exclusively in the subpopulation of isolates that harbor the resistance genes [97, 102]. Thus, the cells are armed with the IS elements needed to activate expression of the resistance gene(s) that they harbor.

The rate of antibiotic resistance in anaerobic bacteria will undoubtedly continue to increase. The existence of populations of *Bacteroides* harboring silent antibiotic resistance genes that can be activated by the simple insertion of an IS element within the promoter bodes an ominous forecast for the rate at which resistance may emerge given selection pressure.

The increasing threat of antibiotic resistance necessitates the development of new antimicrobial agents. Elucidation of the molecular mechanism(s) of resistance is critical to the design of effective new drugs. Strains harboring defined resistance(s) can be used to screen potential new compounds for their effectiveness against preexisting resistances. Knowledge of the active site of a given resistance protein will permit the modeling of novel chemical structures and modification of known active compounds to create new agents with activity against resistant organisms. It is hoped that such efforts will yield new antimicrobial agents that will be effective as treatment of infections caused by resistant bacteria, both anaerobic and aerobic.

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