Prevalence of Antibiotic Resistance in Anaerobic Bacteria: Worrisome Developments

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Antibiotic-resistant anaerobic bacteria have become increasingly recognized as a confounding factor in the selection of therapeutic agents. The use of potent, broad-spectrum antibiotics as empirical therapy, along with appropriate adjunctive measures, has, in some ways, masked the magnitude of the antibiotic resistance problem that parallels that observed for nonanaerobic pathogens. The use of standardized testing methods that recognize resistance and an understanding of resistance mechanisms have become essential for the treatment of patients and the development of new agents.

Antibiotic resistance among anaerobes has steadily increased since the early 1970s. The most frequently isolated antibiotic-resistant anaerobe is *Bacteroides fragilis*. However, resistance is also seen among anaerobes that were previously considered to be highly susceptible to antibiotics, raising concerns about appropriate empirical therapy. Although resistance trends have been monitored and reported predominantly through national and local surveys, susceptibility testing of anaerobic bacteria at individual hospitals remains uncommon. A recent report demonstrating an association between antibiotic-resistant *B. fragilis* and adverse outcomes has prompted new recommendations for susceptibility testing of these organisms [1]. The increase in antibiotic resistance among anaerobes has spawned extensive investigation into the mechanisms of resistance and resistance-gene transfer. These studies have begun to provide insights into why resistance has emerged so rapidly and why it is of concern.

INFECTIONS INVOLVING ANAEROBES

Clinically, antibiotic resistance among anaerobic bacteria can go unnoticed for several reasons, predominantly because many mixed infections involving anaerobic bacteria respond to debridement or drainage. In addition, the pharmacokinetics and penetration of antibiotics to the site, the efficacy of antibiotics against aerobic organisms, and the general health of the patient can also significantly influence outcome. Lastly, inadequate isolation, identification, and susceptibility testing of anaerobes from patients with mixed infections limit analysis and correlation with clinical outcome [2].

Although several retrospective studies have reported a correlation between antibiotic susceptibility among anaerobes and poor outcome, one sentinel prospective observational study of bacteroides bacteremia has reported adverse outcomes in patients receiving an antibiotic to which the organism was not susceptible [1]. Taken together, these studies have been a driving force for increased susceptibility testing recommended in various publications, including NCCLS documents and the *Manual of Clinical Microbiology* [2, 3]. Recent standardization of testing methods by the NCCLS also allows for comparison of resistance trends among laboratories.

SUSCEPTIBILITY TESTING: STATE OF THE ART AND INTERPRETATION

Since 1997, there have been significant changes to the standards for susceptibility testing of anaerobic bacteria [4]. Although there are no automated methods at present, reproducible results can be obtained by 1 of 3 methods [2]. Currently, for surveillance purposes, the NCCLS recommends using the agar dilution method to test \( \geq 100 \) anaerobic isolates on an annual basis at individual hospitals to monitor for resistance trends. Although labor-intensive, this highly reproducible method allows for batch testing of up to 30 isolates of any anaerobe at one time against a single antibiotic.

Results of 2 alternative methods correlate well with the reference standard. Broth microdilution panels provide a convenient, user-friendly method to simultaneously determine sus-
susceptibilities of a single isolate to several antibiotics. Results using this method have had good correlation with those of the agar dilution standard for organisms that grow well in broth supplemented with NCCLS-recommended Brucella blood agar. The ability of either method is now considered to be equivalent for determining antimicrobial susceptibilities of B. fragilis group isolates, but the degree of correlation of results for nonbacteroides anaerobes is not satisfactory. Therefore, NCCLS does not recommend this method for nonbacteroides anaerobes at this time, although it can be used if the testing laboratory validates results against the agar dilution standard. The second user-friendly method is the Etest (AB Biodisk), a simple gradient method that provides accurate results but is limited to a single isolate-antibiotic susceptibility test per strip. Both alternative methods are well suited for testing individual isolates when indicated, such as when results are needed to select therapy on the basis of positive blood culture results, persistent infection, or known resistance of a particular anaerobic species. Organisms that should be considered for individual isolate testing include highly virulent pathogens for which susceptibility cannot be predicted, such as Bacteroides, Prevotella, Fusobacterium, and Clostridium species, Bilophila wadsworthia, and Sutterella wadworthensis [2, 3].

Interpretation of susceptibility results must also be considered when choosing an antibiotic. An MIC determined using any of the above methods does not represent an absolute value. Instead, the “true” MIC is somewhere between the resultant MIC and the next-lower or -higher test concentration. Furthermore, all dilution-based susceptibility methods allow for 2-fold differences on successive testing [2]. The phenotypic interpretation of results of MIC tests (i.e., sensitive [S], intermediate [I], and resistant [R]) are based on the MIC distribution of the bacterial population, the pharmacokinetics and pharmacodynamics of the antibiotic, and the verification of antibiotic efficacy by clinical studies. These S, I, and R breakpoints are established independently by the NCCLS and the US Food and Drug Administration (FDA). For infections involving anaerobes, treatment should include maximum doses of an antibiotic when MICs are at or near the S or I breakpoint, because of the lower penetration and stability of the agent at the site of most infections [2].

TRENDS AND MECHANISMS OF ANTIBIOTIC RESISTANCE AMONG ANAEROBIC BACTERIA

Results of antimicrobial susceptibility tests reported in the literature can vary widely according to the methods and media used, the study size (single hospital vs. multihospital), the geographic region, and antibiotic pressure. Longitudinal surveys using the same methodology may provide the most useful general information about antibiotic susceptibility among anaerobes for the clinician, in the absence of on-site testing of each individual isolate. The majority of results cited in this article for individual antibiotics were from large established surveys that used identical or validated methods. Antibiotics discussed below comprise the most commonly used agents for treating anaerobic infections in humans.

Clindamycin resistance. Although clindamycin is considered to be a gold standard for treatment of anaerobic bacterial infection since the 1960s, antibiotic resistance to clindamycin among these pathogens has increased steadily over the past 15 years. The national anaerobe survey performed by Tufts–New England Medical Center (Boston) has reported frequencies of resistance among anaerobes in the B. fragilis group as low as 3% in 1987, with increases to 16% and 26% in 1996 and 2000, respectively [5–7]. Individual medical centers in these and other studies have found frequencies of resistance to clindamycin to be as high as 44%. Therefore, the results at one medical center may not be predictive of those at another [8]. Resistance to clindamycin among nonbacteroides anaerobes, such as Prevotella, Fusobacterium, Porphyromonas, and Peptostreptococcus organisms, is generally much lower and often <10% [9]. However, longitudinal survey data on nonbacteroides organisms is limited, so trends are not readily identifiable. Among all anaerobes, Clostridium difficile is the most resistant to clindamycin, with as many as 67% of isolates showing resistance [10].

Several genetic clindamycin-resistance determinants have been identified in the B. fragilis group of organisms (ermF, ermG, and ermS), Clostridium perfringens (ermQ and ermP), C. difficile (ermZ, ermB, and ermBZ), and Porphyromonas, Prevotella, Peptostreptococcus, and Eubacterium species (ermF) [11]. For both B. fragilis organisms and C. difficile, these determinants can be located on the chromosome, plasmids, or transposons and are transferable by conjugation. Resistance is mediated by a macrolide-lincosamide-streptogramin type 23S RNA methylase, similar to that in staphylococci [12]. The genes generally encode high-level resistance and are driven by strong promoters. However, not all clindamycin-resistant bacteroides harbor erm genes, and alternative mechanisms for a minority of strains are likely [13]. Of importance, in vitro transfer of ermF between bacteroides was first described in 1979, with some studies demonstrating a frequent cotransfer of tetracycline resistance [14–16].

With the rapid increase in the prevalence of clindamycin resistance, particularly among organisms in the B. fragilis group, this agent is no longer considered to be first-line therapy for infections involving these organisms [17]. Clindamycin can still be considered when treating bacteroides with known susceptibilities or other mixed infections that do not harbor or are not likely to harbor these bacteria, such as oral infections or aspiration pneumonia.

β-lactam resistance. β-lactam agents provide an important therapeutic role in the treatment of infections involving
anaerobes, although significant resistance to some of these agents has also been reported. Among anaerobes, the *B. fragilis* group of organisms have the highest prevalence of resistance to β-lactams. Nearly all (>97%) of the organisms in the *B. fragilis* group are resistant to penicillin G. In contrast, the cephamycins cefoxitin and cefotetan have much better activity against members of the *B. fragilis* group, although the prevalence of resistance among these organisms has been increasing. During 1987–2000, resistance to cefotetan was observed in 8%–14% of isolates overall, according to results reported for the *B. fragilis* group [5, 7]. Significant variation was again seen among individual medical centers, with resistance in 22% of isolates at one site [5]. Cefotetan has similar activity to that of cefoxitin for *B. fragilis*, but it is much less active against other members of the *B. fragilis* group (with resistance rates of 30%–87%, depending on the species). This high prevalence of resistance and interhospital variation have also resulted in recent recommendations against their use as empirical therapy for intra-abdominal infections [17]. Piperacillin resistance has also increased since the introduction of this agent in the 1980s. Until 1990, the prevalence of resistance was <10%, but it has recently increased to 25% at some medical centers, with significant variability among organisms in the *B. fragilis* group [5, 11]. This agent is also not currently recommended as empirical therapy for intra-abdominal infections.

Among the most active β-lactam agents (i.e., those for which resistance consistently ranges from ≈2% to 5%) are the β-lactam/β-lactamase inhibitor combinations ampicillin/sulbactam, ticarcillin/clavulanate, and piperacillin/tazobactam. According to the most recent national surveillance data, <2% of the strains in the *B. fragilis* group as a whole were resistant in 2000 [5]. However, strains of non-β-lactamase-resistant *Bacteroides distasonis* frequently have higher MICs for all 3 antibiotic combinations, with some additional strains resistant to ampicillin/sulbactam. Among all of the β-lactam agents, the most potent are the 3 carbapenems—imipenem, meropenem, and ertapenem—with <0.2% of isolates in the *B. fragilis* group resistant to these agents worldwide [5, 18, 19].

Resistance to β-lactam agents among nonbacteroides anaerobes is generally much lower than that seen for the *B. fragilis* group, but it can be highly variable. Reports are generally limited to comparative in vitro studies in which these agents are tested against a small number of isolates from individual hospitals. Because these organisms are typically more difficult to isolate and identify, the frequency of testing at individual hospitals is exceedingly low. However, one multicenter study using the broth microdilution method showed that 83% of *Prevotella* isolates were resistant to penicillin G, whereas resistance was much lower for species of *Fusobacterium* (9%), *Porphyromonas* (21%), and *Peptostreptococcus* (6%) [9]. Isolates from all 4 genera were 100% susceptible to cefoxitin, β-lactam/β-lactamase inhibitor combinations, and carbapenems, except for *Peptostreptococcus* isolates (4% of which were resistant to ampicillin/sulbactam) and *Porphyromonas* isolates (5% of which were resistant to cefoxitin) [9].

Resistance to β-lactam antibiotics is mediated by 1 of 3 major resistance mechanisms: inactivating enzymes (β-lactamasess); low-affinity, penicillin-binding proteins; or decreased permeability. Inactivating β-lactamasess are the most common and mediate the most diverse mechanisms of resistance.

The most common β-lactamase found among *Bacteroides* and *Prevotella* species are functional class 2e cephalosporinas [20, 21]. These enzymes are all inhibited by the classical β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam). Thus, whereas penicillin or ampicillin are not very active against most *B. fragilis* and *Prevotella* species, the β-lactam/β-lactamase inhibitor combinations are highly active. Cefoxitin-hydrolyzing proteins, such as those encoded by *cepA* and *cfxA*, although far less common, inactivate cefoxitin and cefotaxime but have been observed in many species in the *B. fragilis* group [22].

Production of β-lactamasess by other anaerobic bacteria has been less well studied, but clostridia (other than *C. perfringens*), *Porphyromonas* species, and fusobacteria express resistance via \( \geq 1 \) of these enzymes. Penicillin-resistant isolates of fusobacteria and clostridia express penicillinases that are typically inhibited by clavulanic acid, although exceptions among some *Clostridium* species have been reported [13, 23].

Anaerobes that produce zinc metallo-β-lactamasess are the most worrisome. These enzymes, encoded by the *ccrA* or *cflA* genes, readily hydrolyze the carbapenems imipenem, meropenem, and ertapenem, as well as all β-lactam agents that have known activity against anaerobes. These β-lactamasess are not inactivated by current β-lactamase inhibitors [24]. Fortunately, although first reported in 1986 in *B. fragilis* [25], this resistance mechanism remains relatively rare. It is important to note that as many as 4% of *Bacteroides* species actually carry the *ccrA* or *cflA* genes, but the proteins are not typically expressed at a sufficiently high level to classify the strains as resistant (≤0.8%) [26]. However, high-level expression of this enzyme can occur following in vitro selection with imipenem in the laboratory [27]. Such imipenem-resistant strains are found to contain insertion sequences, which are mobile genetic elements with divergent promoters inserted immediately upstream of the *ccrA* or *cflA* genes, resulting in increased expression of the enzymes up to resistance levels [28, 29]. Laboratory observations were corroborated with similar findings from imipenem-resistant clinical isolates that also contained this arrangement of insertion sequence and promoter gene [27]. For patients infected with these strains, treatment with antibiotics other than β-lactams may be required.

Penicillin-binding proteins such as PBP 1 complex and PBP
2, although essential to the activity of a β-lactam agent, have not been reported to be major mechanisms of resistance development among anaerobes. Altered PBP 2 or PBP 1 complexes have been reported in rare clinical isolates, resulting in cefoxitin and other cephalosporin resistance in B. fragilis [30], or can be induced in vitro [31]. A third mechanism of resistance is the most recent to be studied and perhaps the least understood. Recent studies of pore-forming proteins of gram-negative anaerobic bacteria include identification and cloning of outer-membrane proteins from bacteroides, Porphyromonas species, and fusobacteria. The absence of >=1 outer-membrane protein has been found to be associated with resistance to ampicillin/sulbactam in some strains reported by Wexler [32].

Although the prevalence of resistance has increased to involve some previously effective antimicrobials, several β-lactam antibiotics remain an important part of an antimicrobial armamentarium, as long as prescribing physicians are familiar with resistance patterns in their own hospital or individual testing is performed. Selective pressure similar to that for many aerobic species likely plays an important role in the development and selection of resistance to this class of agents.

**Metronidazole resistance.** Resistance among gram-negative anaerobic bacteria to metronidazole is rare. Surveys conducted in the United States have not reported resistant strains of B. fragilis with metronidazole MICs >16μg/mL, the breakpoint for resistant organisms. However, resistant strains do exist worldwide, with numerous case reports from several countries in Europe [33, 34]. Metronidazole resistance is more common among gram-positive anaerobic bacteria, including most isolates of Propionibacterium acnes and Actinomyces species, as well as some strains of lactobacilli and anaerobic streptococci [18].

Metronidazole and other 5-nitroimidazoles must be reduced to form the active antibacterial agent, which is stable only under anaerobic conditions. Nitroimidazole resistance genes (nim) have been identified in strains with high MICs of metronidazole (i.e., >4 μg/mL) [35]. nim genes encode a nitroimidazole reductase, which reduces 4- or 5-nitroimidazole to 4- or 5-aminoimidazole and prevents the formation of toxic nitroso residues necessary for the agents' activity [36]. Six related chromosomal or plasmid-based nim genes (nim A–F) have now been reported in Bacteroides species [35]. Insertion sequence elements, either identical or similar to those found in imipenem-resistant strains, are also found upstream of the nim genes, likely increasing their expression [37]. Mechanisms for resistance among nonbacteroides anaerobes is not well understood.

Fortunately, with only few anaerobic isolates resistant to metronidazole, this agent will likely remain a mainstay for combination treatment of mixed infections for the near future. However, some authors warn that, with transferable resistance determinants already identified, an increase in the number of resistant strains under selective pressure may be looming [38].

**Quinolone resistance.** Historically, fluoroquinolones were not considered to be active agents against anaerobic bacteria. However, 2 agents, temafloxacin and trovafloxacin, were approved by the FDA for treatment of infections involving anaerobic bacteria because of their broad-spectrum activity against pathogens such as Bacteroides species and most other anaerobic species. Temafloxacin was used briefly in the early 1990s before its withdrawal because of toxicity. Trovafloxacin was approved in December 1997, but its use was also subsequently severely restricted because of toxicity. Antibiotic surveillance testing between 1994–1996, before the introduction of trovafloxacin, revealed low-level resistance among 3%–8% of organisms in the B. fragilis group [7]. In 1997, resistance was already at 13%, with an additional increase to 15% in 1998, the year in which the drug was launched. Despite the very limited use of trovafloxacin in 1998 and 1999, frequencies of resistance increased further, reaching a peak of 25% in 2001 [39]. Thus, it has been speculated that older fluoroquinolone agents, such as ofloxacin, levofloxacin, and ciprofloxacin, were responsible for this increase in resistance. Of interest, moxifloxacin MICs have also increased [39]. This agent is currently approved by the FDA for a number of nonanaerobic bacteria but is only in phase III trials for mixed infections that include anaerobes.

Resistance to fluoroquinolones among anaerobes may be due to 1 or more concurrent mechanisms. In both aerobic and facultatively anaerobic bacteria, fluoroquinolones inhibit DNA gyrase and topoisomerase IV, both of which are essential for bacterial DNA replication. Resistance in aerobes occurs by mutations in gyrase (gyrA) and topoisomerase IV (parC) genes and/or by increased expression of efflux pumps. Recently, similar mechanisms have been discovered in some anaerobic bacteria. In B. fragilis, both gyrA and gyrB were cloned from the bacteroides genome in 1999 by Onodera and Sato [40]. Using stepwise selection with levofloxacin, these authors reported a mutation at residue Ser-82-Phe of GyrA corresponding to Ser-83-Phe of the quinolone resistance determining region (QRDR) of gyrA, in Escherichia coli. Three mutants with MICs of levofloxacin of 12.5–50 μg/mL had the identical Ser-82-Phe substitutions. Of importance, cross-resistance to sitafloxacin, ciprofloxacin, and sparfloxacin was also seen, as evidenced by elevated MICs of these agents.

Additional studies have shown the same or similar effects of less well-characterized substitutions in the QRDR region on selection for resistance against ciprofloxacin and trovafloxacin in vitro [41] and after oral administration of clinafoxacin to healthy volunteers [42]. Regardless of the selective agent, both studies found higher MICs of all quinolones tested. These findings raise significant concerns about the exposure of anaerobes and other commensal organisms to a common class of oral
antibiotics and the potential for developing resistance to antibiotics currently under development.

In addition to QRDR mutations, efflux pumps mediate the second mechanism of resistance to quinolones in aerobic and facultative anaerobic bacteria, and likely in the anaerobes as well. Oh et al. [43] and Ricci and Piddock [44] have both recently identified ≥1 efflux pumps in *B. fragilis*, with potentially overlapping substrates, that confer resistance to both fluorinated and nonfluorinated quinolones. The potential combination of mutations in the QRDR region with efflux pumps poses significant hurdles to the long-term efficacy of any agent in this class.

**TRANSFER OF ANTIBIOTIC RESISTANCE**

A discussion of antibiotic resistance mechanisms would not be complete without reference to the importance of horizontal dissemination of antibiotic resistance genes within and from anaerobic species. Transfer of resistance genes has been described for anaerobes in the *B. fragilis* group and in *Prevotella, Clostridium*, and *Fusobacterium* species [11].

Bacterial conjugation appears to be the preferred method of transfer of antibiotic resistance genes. Resistance genes are harbored on transposons, plasmids, and chromosomal elements, many of which are mobile. Many of these elements are also small, carrying only the necessary genes for initiation of DNA transfer. Physical transfer of the DNA from cell to cell requires a mating bridge that appears to be encoded by much larger conjugative transposons, which are themselves transferable [45].

Some features of conjugative transposons raise particular concern about their role in the spread of antibiotic resistance. First, all known conjugative transposons in bacteroides harbor tetracycline resistance genes, determinants that serve as markers for the presence of such transposons. Although tetracycline itself may be of decreasing therapeutic importance, ∼70% of organisms in the *B. fragilis* group are resistant to tetracycline and thus are likely harbor conjugative transposons. The most thoroughly studied conjugative transposon, CTnDOT, contains a tetracycline resistance determinant as well as genes whose products are involved in the formation of the mating bridge [46]. Of particular interest, the rate of transfer of conjugative transposons can increase by several orders of magnitude after exposure of donor strains to subinhibitory levels of tetracycline (i.e., “Tet induction,” which is unrelated to tetracycline resistance). More importantly, upon Tet induction, these strains can and will transfer any coresident mobilizable elements, many of which also carry antibiotic resistance genes. Thus, bacteroides encode an efficient conjugation system in which multiple, different transfer factors use mating-bridge pathways that are inducible [45]. This phenomenon has significant implications for the spread of antibiotic resistance genes among organisms in the Bacteroides genus, as well as other gut commensals [47].

**CONCLUSIONS**

Recognition and control of antibiotic resistance among anaerobes is of increasing importance. Although appropriately heightened attention has been given to resistant aerobic bacteria for several decades, awareness of similar developments among anaerobic bacteria is needed. The consequences of indiscriminate use and overuse of antibiotics against anaerobic bacteria clearly extends beyond the readily identifiable impact on aerobes and warrants redoubling our diligent and prudent use of antibiotics.

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**References**