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Anaerobic Infections
Update on Treatment Considerations

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Abstract

Anaerobic bacteria are the predominant indigenous flora of humans and, as a result, play an important role in infections, some of which are serious with a high mortality rate. These opportunistic pathogens are frequently missed in cultures of clinical samples because of shortcomings in collection and transport procedures as well as lack of isolation and susceptibility testing of anaerobes in many clinical microbiology laboratories. Correlation of clinical failures with known antibacterial resistance of anaerobic bacteria is seldom possible. Changes in resistance over time, and the discovery and characterization of resistance determinants in anaerobic bacteria, has increased recognition of problems in empirical treatment and has even resulted in changes in treatment guidelines. This review discusses the role of anaerobic bacteria in the normal flora of humans, their involvement in different mixed infections, developments in antibacterial resistance of the most frequent anaerobic pathogens and possible new treatment options.

Anaerobic bacteria are important pathogens in many different types of infections and may be involved in a special way in certain other processes as well, such as bacterial overgrowth syndrome. The indigenous flora of the body is the principal reservoir of these organisms, but some clostridial infections may have an exogenous source. The spectrum of severity of anaerobic infections varies from mild to severe and life-threatening. Although there are currently many

antimicrobial agents with good activity against anaerobic bacteria, more and more knowledge has accumulated about the development of resistance against these drugs in anaerobic bacteria. While clinicians and microbiologists are more knowledgeable about anaerobic bacteria and their role in disease than was true 1 or 2 decades ago, there has been a real tendency recently to do fewer anaerobic cultures and to minimize the workup (identification and resistance determination) on specimens that are cultured for these organisms. This is related to several issues, such as availability of generally effective antimicrobials, the time it takes for useful information about the infecting flora to be generated in the laboratory and economic considerations. The fully empirical usage of highly potent drugs to treat mixed infections involving anaerobes leads, predictably, to increased resistance to these drugs among anaerobes. The aims of the present review are to summarize the composition of the indigenous anaerobic flora of the human body, discuss the most prevalent anaerobic bacteria in different infections occurring in various body sites, give an overview of the most important resistance mechanisms of the different groups of anaerobic bacteria that lead to increased levels of resistance to anti-anaerobic drugs, and summarize the new treatment options available today.

1. Anaerobic Bacteria as Members of the Normal Flora

Anaerobic bacteria are a major component of the human microflora, where they reside on mucosal membranes. In a number of areas they outnumber aerobes by a factor of 10 : 1 to 10 000 : 1 (table I). Through the use of special anaerobic culturing methods or molecular genetic procedures, several hundred species of anaerobic organisms have been identified in the human indigenous flora.[1,2] They predominate in many infectious processes, particularly those arising from mucosal sites. The composition of the normal flora and the number of anaerobic species present at different sites of the body are important from several aspects. Information concerning what organisms make up the indigenous flora allows the anticipation of the presence of certain organisms, in particular specimens taken from infectious processes, which assists the clinician in the choice of suitable drugs for initial therapy. A knowledge of the normal flora in various regions may also allow a readier judgement as to whether a given isolate is significant or not, as in the case of *Propionibacterium* spp. isolated from blood cultures. The three major sites of normal colonization of mucosal surfaces by anaerobic bacteria are the oral cavity, the intestinal tract and the female genital tract.

The composition of normal flora with regard to the anaerobic species present at the different sites can differ significantly.[3] *Bacteroides thetaiotaomicron*, *Bacteroides fragilis* and other members of the *Bacteroides* genus, together with clostridia and anaerobic cocci, are more frequently isolated from intestinal tract specimens, whereas *Prevotella bivia*, *Prevotella disiens* and other pigmented species of *Prevotella* and *Porphyromonas* are the predominant Gram-negative anaerobic bacilli in the normal vaginal flora. Hydrogen peroxide-producing lactobacilli and peptostreptococci are also important constituents of the normal flora of the vagina. A very complex indigenous anaerobe flora is to be found in the oral cavity; it changes in composition with age, with the anatomical location of the sampling and with the level of oral hygiene.[4] Anaerobic Gram-positive cocci together with the *Prevotella oris*, *Prevotella buccae* and *Prevotella oralis* group, pigmented *Porphyromonas* species and fusobacteria (*Fusobacterium nucleatum* and *Fusobacterium necrophorum*) are the most frequently isolated anaerobic species in the normal flora of the oral cavity. The skin is a less important site in this respect, and the anaerobes present there (propionibacteria and anaerobic cocci) are more often regarded as contaminants in different clinical samples.

The occupation of distinct ecological niches within the intestinal environment by the great number of anaerobic bacteria effectively interferes with colonization by potentially pathogenic bacterial species through the depletion of oxygen and nutrients, and the production of enzymes and toxic end-products. This protective process is termed colonization resistance. The production

Table I. The most frequent anaerobic genera as members of the normal flora at different body sites

Anaerobic bacteria	Skin (10 : 1)[a]	Oral cavity (1 : 10)[a]	Intestine (1 : 1000–1 : 10 000)[a]	Vagina (1 : 10–1 : 100)[a]
Gram-positive rods				
Actinomyces	–	+	+	+
Bifidobacterium	–	+	+	+
Clostridium	–	–	+	+
Eubacterium	–	+	+	+
Lactobacillus	–	+	+	+
Propionibacterium	+	+	+	+
Gram-positive cocci				
Coprococcus	–	–	+	–
Gaffkya	–	–	+	–
Peptococcus	+	–	+	+
Peptostreptococcus	+	+	+	+
Ruminococcus	–	–	+	–
Sarcina	–	–	+	+
Microaerophilic streptococci	+	+	+	+
Gram-negative rods				
Bacteroides	–	+	+	+
Porphyromonas	–	+	+	–
Prevotella	–	–	+	+
Butyrivibrio	–	–	+	–
Desulfonomas	–	–	+	–
Fusobacterium	–	+	+	+
Leptotrichia	–	+	+	–
Selenomonas	–	+	(?)	–
Succinimonas	–	–	+	–
Succinivibrio	–	–	+	–
Wolinella	–	+	+	–
Gram-negative cocci				
Acidaminacoccus	–	–	+	+
Megasphera	–	–	+	–
Veillonella	–	+	+	+
Spirochetes				
Treponema	–	+	(?)	+
Other spirochetes	–	(?)	+	(?)

a Ratio of aerobes : anaerobes.

– indicates not found or rare; + indicates usually present; **(?)** indicates that its presence is questionable.

of vitamin K by anaerobes in the intestines is beneficial to the host, while the production of bile by these organisms is useful in fat absorption and cholesterol regulation. On the other hand, members of the normal flora, both aerobes and anaerobes, may also be reservoirs of resistance genes, and the very active transfer of these genes among the same or other species may occur.[5]

2. Role of Anaerobic Bacteria in Infectious Processes

Despite the high number of anaerobic species present in the normal flora, relatively few are involved in human infections. Many anaerobes behave as opportunistic pathogens when they encounter a permissive environment within the

host. These organisms generally cause disease subsequent to breakdown of the mucosal barriers, and the indigenous flora penetrate into normally sterile body sites. Very few major syndromes are due to exogenous infections by anaerobes (mostly clostridia). Most often, anaerobes are found as part of a mixed flora, although solitary infections also occur.[1] The spectrum of severity of anaerobic infections varies from life-threatening clinical cases to mild infections. Characteristically, anaerobic infections are characterized by tissue necrosis and suppuration. Some anaerobic bacteria may resist oxygenated microenvironments, and synergy with other aerobic or facultative bacteria is also well known.[1]

The success of the clinical microbiology laboratory to isolate clinically significant anaerobic bacteria from a monobacterial or mixed infection is highly dependent on how the sample has been taken: the sample should be protected from the contamination with normal flora, and special anaerobic containers should be used for specimen collection and transport. Rapid processing of samples is also important in avoiding overgrowth by facultative anaerobes. Even though most anaerobic bacteria of clinical importance are oxygen tolerant, laboratories need to provide proper anaerobic environment for primary culture and subculture of more fastidious anaerobic bacteria as well.[1,6]

After the contamination of previously sterile sites by mucosal microflora, the relatively few anaerobic bacteria that survive at the infected site are those that have resisted changes in oxidation-reduction potential and host defence mechanisms (table II). The hallmark of infection caused by the most frequently isolated Gram-negative anaerobic bacteria is abscess formation, although in some cases they may reach the bloodstream and cause sepsis.[6,7] Abscess formation is located typically at sites of direct bacterial contamination (very often in consequence of the presence of *B. fragilis*), although distant abscesses resulting from haematogenous spread are not uncommon with the more virulent anaerobes.

Approximately 5% of bacteraemia cases involve anaerobic bacteria alone or together with aerobes. *B. fragilis* is the anaerobe most

Table II. Major sites of anaerobic infections

Clinical picture
Bloodstream infection
Infections of head and neck
brain abscess
extradural or subdural empyema
chronic sinusitis
chronic otitis media
wound infections after head and neck surgery
ocular infections
dental and oral infections
peritonsillar abscess
periodontitis
root canal infection
periodontal abscess
Thoracic infections
aspiration pneumonia
necrotizing pneumonia
lung abscess
empyema (nonsurgical)
Intra-abdominal infections
postsurgical intra-abdominal infections
liver abscess
appendicitis
peritonitis
biliary tract infections
Enteric diseases
Clostridium difficile infection
enterotoxin-producing *Bacteroides fragilis* infection
Obstetric-gynaecological infections
septic abortion
vulvovaginal abscess
tubo-ovarial and pelvic abscess
endometritis
postoperative wound infection
bacterial vaginosis
Skin and soft-tissue infections
gas gangrene (anaerobic myonecrosis)
bite infections
animal bites
human bites
diabetic foot infection
connective tissue and soft tissue abscess
decubitus ulcer
perirectal abscess
breast abscess

commonly isolated from these infections.[1,6,8] Anaerobic bacteraemia is usually secondary to an infection started from the bowel, female genital tract, respiratory tract, soft tissue or oral cavity, such as in the case of Lemierre's syndrome involving thrombophlebitis of the jugular vein.[9] Transient bacteraemia involving anaerobes may occur spontaneously or may follow manipulation of various body sites, such as the oral cavity. Such transient bacteraemia may follow vigorous dental prophylaxis, endodontic therapy as well as extractions. Recovery of anaerobic bacteria from the bloodstream requires the consequent use of anaerobic blood culture bottles in septic patients.[7]

Infections caused by anaerobes in the intra-abdominal region generally occur on disruption of the commensal relationship with the host. Anaerobic Gram-negative bacilli (members of the *B. fragilis* group) that reside in the intestine can cause infection together with aerobes following contamination of normally sterile sites, such as the peritoneal cavity, by caecal contents. In these infections, anaerobes greatly outnumber aerobes, and *Escherichia coli* is the predominant aerobic or facultative organism. Other anaerobes that are commonly isolated from this type of infection include *Peptostreptococcus micros*, *Prevotella intermedia* and *Fusobacterium* spp. The involvement of *Clostridium* spp. can lead to more severe infection.[1,3]

Severe infections of the head and neck may arise from an abscessed tooth infected with the commensal microflora of the mouth. Other infections of the oral cavity, such as periodontitis and necrotizing gingivitis, are closely correlated with poor oral hygiene. The anaerobes that predominate in these processes are *Porphyromonas gingivalis* and *Prevotella melaninogenica*. Anaerobic infections that cause abscess formation in the pharynx can lead to supportive thrombophlebitis of the jugular vein; a syndrome may develop, occasionally with a fatal outcome, known as Lemierre's syndrome. It is characterized by bacteraemia and septic emboli to the lungs, brain and heart. *F. necrophorum* is a common cause of these infections; it has tended to be forgotten during the past 60 years, but currently there are increasing numbers of reports of cases that were not recognized in time.[9] Chronic otitis and chronic sinusitis may also be caused by a mixture of aerobic and anaerobic bacteria, where *Bacteroides* group members predominate as the anaerobic component of the pathogenic flora.[10] In brain abscesses, *Fusobacterium*, *Bacteroides* and Gram-positive anaerobic cocci (GPAC) predominate. They may arise by haematogenous dissemination from a distant infected site or by direct extension from chronic otitis or sinusitis, or may be due to a dental infection. Pulmonary infections in which anaerobic bacteria predominate are most commonly associated with the aspiration of oropharyngeal material or occur as a complication of periodontal disease. The most common anaerobes in these infections are indigenous to the upper airways and the oral cavity, and include *Bacteroides*, *Prevotella*, *Fusobacterium* and *Peptostreptococcus* spp. Four major syndromes can develop: aspiration pneumonia, necrotizing pneumonia, lung abscess and empyema.[11]

Anaerobes originating from the vaginal flora are encountered in pelvic abscesses, septic abortion, endometritis, tubo-ovarian abscess, pelvic inflammatory disease and postoperative infections. The major isolates from these infections are *B. fragilis*, *P. bivia*, *P. disiens*, *P. melaninogenica,* peptostreptococci and *Clostridium* spp. As in intra-abdominal infections, most infections of the female genital tract are mixed, involving both anaerobes and aerobes. However, infections in which anaerobes are isolated in pure culture occur more frequently in the pelvis than in the abdominal cavity.[12] Bacterial vaginosis, a disease process in which anaerobes predominate together with *Gardnerella vaginalis*, is characterized by a malodorous discharge. The overgrowth of a mixture of anaerobic bacteria including *Prevotella* spp., *Mobiluncus* spp. and peptostreptococci proceeds in parallel with the disappearance of the normal lactobacillus flora.

Anaerobic infections of the skin and soft tissues are most often caused by contamination with faecal or oral flora. Diabetic foot ulcer is one of the specific forms of skin infections. Anaerobic bacteria are involved usually in grade 2 or 3 of the

disease, if the wound is ischaemic and infected at the same time.[13] The presence of the anaerobic bacteria in the mixed flora can be proven only if a sample is taken from below the surface of the wound.[3] Infections of the skin or soft tissue after a human or animal bite involve the indigenous anaerobic flora of the oral cavities.[14] It is important to isolate and report the presence of anaerobes in these infections because the choice of antimicrobial therapy should be based on the susceptibility of anaerobes as well. Dog bites may include a crush injury, and through the introduction of dog oral flora more than half of such wound infections harbour anaerobic bacteria. Most frequent isolates include *Prevotella heparinolytia*, *Bacteroides pyogenes*, *Bacteroides tectus* and animal species of *Porphyromonas*.[14] Anaerobes can also be found in necrotizing fasciitis, usually as part of a mixed anaerobic/aerobic infection. The disease can spread rapidly and be very destructive, with gas formation in the infected tissues. *Peptostreptococcus* and *Bacteroides* spp. usually predominate together with clostridia.[15]

Anaerobic infections of bones and joints, such as osteomyelitis or septic arthritis, are rare, but typically arise from infected adjacent soft tissue sites. *Fusobacterium* species are the most common Gram-negative anaerobes isolated from infected joints, whereas infected bones may yield a wide variety of isolates. In a recent study involving molecular techniques in diagnostics, 13 patients with prosthetic joint infections due to *Finegoldia magna* (2% of all patients evaluated) were found.[16] Patients presented with either polymicrobial infection after an open fracture or nosocomial infection after recent prosthesis implantation. It was emphasized by the authors that recommended antibacterial prophylaxis has poor activity against *F. magna*.[16]

Diarrhoea is one of the most common complications associated with antibacterial therapy, and colitis is a serious consequence. Since the late 1980s, toxin-producing *Clostridium difficile* has been recognized as the most frequent cause of antibacterial-associated diarrhoea and of pseudomembranous colitis. Moreover, it is accepted that *C. difficile* is the primary cause of nosocomial diarrhoea in hospitals in industrialized countries. With the recent emergence of some highly virulent variants of this pathogen, which are resistant to the frequently used antibacterials such as fluoroquinolones, the importance of the diagnosis and treatment of this infection in hospitals and in the community has increased.[17,18] In case-controlled studies, inflammatory diarrhoea in children and adults with undiagnosed diarrhoeal diseases has been associated with the presence of enterotoxigenic *B. fragilis* (ETBF).[19,20] In an animal model it has been shown that ETBF and non-toxigenic *B. fragilis* both chronically colonize mice, but only ETBF triggers colitis and strongly induces colonic tumours in multiple intestinal neoplasia mice.[21]

3. Trends in Antibacterial Resistance among Anaerobic Bacteria

Anaerobes, and especially the most frequently isolated *B. fragilis* and related species, are inherently resistant to various antibacterials, such as aminoglycosides, first- and second-generation fluoroquinolones and monobactams, and they may additionally exhibit acquired resistance to other agents. Since the early 1970s, there has been a steady increase in resistance among anaerobes against antibacterials previously thought to be effective alone or in combination for treatment of anaerobic infections. The most frequently isolated antibacterial-resistant anaerobes are *B. fragilis* and related species. However, resistance has also emerged among anaerobes earlier considered to be highly susceptible to antibacterials, and this has given rise to concerns about appropriate empirical therapy.

The antimicrobial susceptibility testing of anaerobes is rarely performed at most medical centres in view of the long and special incubation circumstances needed for the isolation, identification and antibacterial susceptibility testing of most anaerobic bacteria, and also for budgetary reasons.[22] Accordingly, antibiogram-guided therapy is rarely available for the treatment of patients. Clinically, antibacterial resistance among anaerobic bacteria can go unnoticed for several

reasons, predominantly because many mixed infections involving anaerobic bacteria respond to debridement or drainage. On the other hand, the efficacy of antibacterials against the aerobic organisms involved in the infectious process and the general health of the patient can significantly influence the outcome. The inadequate isolation, identification and susceptibility testing of anaerobes from patients with mixed infections often limit the analysis and correlation with the clinical outcome. The role of a resistant anaerobe in this setting is difficult to determine. Several retrospective and a few prospective studies have revealed a correlation between clinical failure and the antibacterial resistance of clinically important anaerobes.[7,23-25]

The selection of antibacterials for the empirical treatment of pure or mixed anaerobic infections is very often based on the surveillance data obtained at a local or national level. The Clinical and Laboratory Standards Institute (CLSI) recently established rigorously standardized methodology for the susceptibility testing of anaerobes for minimum inhibitory concentration (MIC) determination by the agar dilution method.[26] The micro-broth dilution method has been validated only for *B. fragilis* group strains, and not for other slow-growing, more fastidious anaerobes. The US FDA-approved E-test® (AB Biodisk, BioMerieux) provides an agar-based gradient method validated against the agar dilution method for anaerobes as well. The disk diffusion test is not accepted for the antibacterial susceptibility determination of anaerobes. The resistance breakpoints set by the CLSI for anaerobes are used worldwide. However, the European Committee on Antibiotic Susceptibility Testing (EUCAST) recently started to collect MIC data for anaerobic bacteria as well, and set resistance breakpoints for most antibacterials for anaerobes. In some cases (such as piperacillin/tazobactam and metronidazole) these differ from those accepted by the CLSI.[26,27] The Europe-wide surveillances carried out by the European Society of Clinical Microbiology and Infectious Disease (ESCMID) Study Group on Antibiotic Resistance of Anaerobic Bacteria (ESGARAB) have demonstrated different tendencies in resistance of some anaerobes in various geographic areas.[28-31]

4. Resistance Problems Relating to Gram-Negative Anaerobes

4.1 *Bacteroides fragilis* Group Strains

Different surveys have shown *B. fragilis* to be more susceptible than other members of the *Bacteroides* genus. Resistance to penicillins is universally high among isolates from the *B. fragilis* group. More than 95% of all species are resistant to benzylpenicillin (penicillin G) and ampicillin, <50% are susceptible to ticarcillin and around 70% are susceptible to piperacillin.[32-34] The common mechanism of resistance to penicillin and ampicillin involves a class 2e cephalosporinase, coded by the chromosomal *cep*A gene.[35] Fortunately, this nearly ubiquitous *Bacteroides* β-lactamase is inhibited by all parenteral β-lactam/β-lactamase inhibitor combinations (ampicillin/sulbactam, amoxicillin/clavulanic acid, ticarcillin/clavulanic acid and piperacillin/tazobactam), although concentration-dependent differences in inhibitory activity among the three β-lactamase inhibitors were observed during investigations of purified β-lactamases obtained from *B. fragilis* or *Bacteroides levii* (recently reclassified as *Porphyromonas levii*).[36] Resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam varies between 2% and 11% in different studies.[32,37] According to the third *Bacteroides* resistance surveillance study carried out in Europe,[38] during the last 2 decades, the resistance of all *Bacteroides* strains to amoxicillin/clavulanic acid has increased from 1% to 10.4%, and that to piperacillin/tazobactam from <1% to 10.1%. *B. fragilis* tends to be more susceptible to both antibacterials than other species of the *Bacteroides* genus.[37]

Of the available cephalosporins and cephamycins with reported activity against *B. fragilis* group infections, cefoxitin remains the most active, with 80–90% of isolates susceptible, followed by cefotetan, although the latter is much less active against non-*B. fragilis* members of the group,[37,39] with a difference for instance of 19% resistance for *B. fragilis* and 72% resistance to non-*B. fragilis* isolates in France.[40] First- and third-generation cephalosporins have poor *in vitro*

activity against nearly all members of the *B. fragilis* group. Resistance to cefoxitin is coded by the *cfx*A β-lactamase gene, which is transferable via the mobilizable transposon (MTn4555).[41] During the past 20 years a very marked increase in resistance to cefoxitin has been observed in Europe, with resistance rates of 3%, 6% and 16.8% in 1988–9, 1999–2001 and 2008–9, respectively.[28,30,38]

Carbapenem resistance has fortunately remained rare among *Bacteroides* strains, accounting for <1% in most countries; however, in some European countries, such as France, the level of imipenem resistance varied considerably between 1992 and 2000 (0–4.4%), but without any special trend in time.[40] During the different ESGARAB surveillances, carried out at three different time points, carbapenem resistance was found to be low at the resistance breakpoint ≥16 μg/mL: 0%, 0.8% and 1.2%, respectively, with quite a few strains belonging in the intermediate category.[28,30,38] There are considerable differences between various geographical areas in this respect. During a national survey in the US, the overall resistance to imipenem and meropenem was 0.5% and 1.0%, respectively.[42] On the other hand, a much higher percentage of resistance to carbapenems was recently reported from Taiwan in the case of blood culture isolates: 7% and 12% of *B. fragilis* isolates (n = 60) and 7% and 3% of *Bacteroides thetaiotaomicron* isolates (n = 30) were non-susceptible to imipenem and meropenem, respectively.[43] This broad-spectrum β-lactam resistance is coded by the *cfi*A (also known as *ccr*A) gene, expressing a class B metallo-β-lactamase that confers resistance to all β-lactam antibiotics, including the β-lactam/β-lactamase inhibitor combinations. These metallo-β-lactamases have been shown to be polymorphous at some amino acid positions.[44,45] For the expression of this gene, special insertion sequences (IS) are needed immediately upstream of the *cfi*A gene to act as a promoter. Different studies have revealed the presence of the *cfi*A gene much more frequently (between 2.4% and 6.9%) on the chromosome of *Bacteroides* strains, with or without the presence of the appropriate IS elements.[46-49] It has also been confirmed that *B. fragilis* strains harbouring the *cfi*A gene may be present in faecal samples of healthy people or those with diarrhoea.[47,50] Two alternative, non-β-lactamase-mediated resistance mechanisms are known for *Bacteroides* strains: (i) alterations of the penicillin-binding proteins (PBP 1 and PBP2); or (ii) porin mutations, which can result in a decreased susceptibility to β-lactam drugs.[51-53]

Clindamycin resistance among *B. fragilis* group strains has become widely prevalent over the last 2 decades, with resistance ranging now between 10% and 40% in many surveillances worldwide.[30,32,54,55] The resistance levels can differ between species: 30% of *Bacteroides vulgatus* and 25% of *Bacteroides ovatus* strains proved resistant to clindamycin, in contrast to only 13% of *B. fragilis* strains.[30] In a recent study, clindamycin resistance also differed geographically for all *Bacteroides* isolates, being 18% in northern Europe and 54% in southern Europe.[38] High-level resistance to clindamycin is mediated by a macrolide-lincosomide-streptomycin B (MLS_B)-type mechanism via 23S rRNA methylases coded by different *erm* genes. However, not all clindamycin-resistant *Bacteroides* strains harbour *erm* genes; alternative mechanisms are likely for a minority of the strains.[56] Transfer of the clindamycin resistance genes together with tetracycline resistance has been demonstrated between *Bacteroides*.[57]

Metronidazole (5-nitro-imidazole) resistance remains rare (using the CLSI resistance breakpoint ≥32 μg/mL) among *Bacteroides* spp., despite its widespread use for prophylaxis and treatment. Resistance to metronidazole has been reported from Asia, Africa, India, several European countries and the Middle East, but very few isolates in the US have been found with MIC >16 μg/mL.[42,58-62] Resistance to metronidazole is most commonly expressed by one of the seven known *nim* genes (A–G) that encode nitroimidazole reductases. For the expression of the *nim* genes, IS elements are also needed as promoters. In a large Europe-wide study, 2% of 1502 *Bacteroides* isolates exhibited decreased susceptibility to metronidazole and harboured one of the *nim* genes. High-level resistance was easily induced in *nim*-positive *Bacteroides* strains belonging to different species by culturing in the

presence of a subinhibitory concentration of metronidazole.[63] Non-*nim* gene-associated metronidazole resistance has also been reported, usually following the extensive use of metronidazole in a given patient. The exact mechanism of this resistance is not known, but induction of the resistance can be observed in the presence of metronidazole *in vitro*.[63,64]

Unlike the earliest fluoroquinolones, several more recent compounds such as moxifloxacin have exhibited good *in vitro* activities against clinically important anaerobes, including *Bacteroides* strains. Moxifloxacin has been improved by the FDA and the European Medicines Agency for the treatment of complicated skin and skin structure infections. Moxifloxacin has a very good, but incomplete, *in vitro* activity against a broad range of anaerobes; nonetheless, a rapid increase in the level of resistance was observed for *B. fragilis* group isolates in different studies in the US and Europe. The resistance to moxifloxacin in Europe has increased from 9% 10 years ago to 15% in a recent study.[30,38] Differences are also observed in the resistance rates when the *Bacteroides* strains from northern Europe (29.9% resistance) are compared with those from southern Europe (7.6%).[38] High-level resistance has been measured during many different surveillances worldwide, with dramatic increases in some places with time.[37,65,66] The most expressed increase in fluoroquinolone resistance was observed by Golan et al.[66] when they followed the changes in resistance between 1994 and 2001 in isolates obtained from 12 US hospitals. Moxifloxacin resistance increased from 30% to 42% (resistance breakpoint 4 μg/mL) between 1998 and 2001 in addition to the increase of resistance to another fluoroquinolone, trovafloxacin, of 8–25% between 1994 and 2001 (resistance breakpoint 8 μg/mL). Increased resistance was observed for all *Bacteroides* species, for all sites of isolation and for 11 of 12 hospitals. The association between increased resistance and year of isolation remained significant after adjustment for hospital, species and site of infection; however, the highest rates of resistance were observed among blood culture isolates. Differences in the rates of moxifloxacin resistance in various studies carried out in the same period of time in different locations may be explained by the different level of usage of older fluoroquinolones (ofloxacin, ciprofloxacin) in hospitals or in the community, which select cross-resistance with newer fluoroquinolones.[37,65,66] Resistance to moxifloxacin among *Bacteroides* spp. is associated with mutations in the *gyr*A and *gyr*B genes, but the increased expression of efflux pumps, either together with the previous mechanism or alone, may also be responsible for the resistance.[67,68] Transferable fluoroquinolone resistance has not been described among anaerobic bacteria.

Tetracycline resistance is widespread among *B. fragilis* group strains; of the three known tetracycline resistance mechanisms (efflux-mediated resistance, enzymatic degradation and ribosomal protection); however, only the latter is operative in members of the *B. fragilis* group, due to the presence of the *tet*Q, or less frequently of the *tet*M or *tet*36 genes.[69] Tigecycline is a recently developed derivative of minocycline, representing a new class of antimicrobials, the glycylcyclines, with excellent activity against anaerobes, including *Bacteroides* strains.[70-72] With the resistance breakpoint of ≥16 μg/mL accepted by the CLSI, only 1.7% of 824 recent *Bacteroides* isolates from different European countries were resistant to this antibacterial,[38] and no resistant strain was found among 522 *Bacteroides* isolates collected during the TEST (Tigecycline Evaluation and Surveillance Trial) study in 2007 and 2008.[71] During a recent study carried out in Belgium[55] using the E-test® methodology, 8% resistance was found among 141 *B. fragilis* group strains using the resistance breakpoint ≥8 μg/mL for tigecycline. In rare cases, Tet(X), a tetracycline-degrading mono-oxygenase, has been found to confer resistance to tigecycline in *Bacteroides* spp.[73]

Table III summarizes data from some geographical areas about the resistance trends of *B. fragilis* group strains.

4.2 Other Gram-Negative Anaerobic Bacilli

Porphyromonas, *Prevotella* (earlier assigned to the *Bacteroides* genus) and *Fusobacterium* species

Table III. Resistance trends in *Bacteroides fragilis* group isolates in different parts of the world

Geographical area	Changes in the percentage of resistance during the past ≥10 years (breakpoints)							References
	AMC (4/16)	PIPT (32/128)	CEF (16/64)	CLIN (2/8)	CARB (4/16)	MET (8/32)	MOXI (2/8)	
Europe	1–10	<1–10	3–16.8	9–31	0–0.8	0–0.5	9–15	28,30,38
US	2–5	2–3	6–22	3–26	<0.8	0–0	23–34	22,32,39,66
France	2.7–10			12–32	0–4	0–5		40
Belgium	4–14	2–7	20–18	17–39	1–0	0–1	32–33	37,55
Spain	5–18	0.7–0.58	13–27	33–48	0.9–0.7	0–0	6–25	70,74

AMC = amoxicillin/clavulanic acid; **CARB** = carbapenem (imipenem or meropenem); **CEF** = cefoxitin; **CLIN** = clindamycin; **MET** = metronidazole; **MOXI** = moxifloxacin; **PIPT** = piperacillin/tazobactam.

are usually considered more susceptible to anti-anaerobic drugs than members of the *B. fragilis* group, but the surveillance data on the susceptibility of these organisms are more limited. Currently, about 50% of *Prevotella* spp. are resistant to penicillin and ampicillin, due to β-lactamase production coded by the *cfx*A gene or some other β-lactamases approximating to those of *Bacteroides*. However, there are some differences in resistance rate between the different species, *P. bivia* having a susceptibility of only 30%, and *P. oris* a susceptibility of 47% according to a European study.[29] They retain susceptibility to piperacillin, piperacillin/tazobactam, amoxicillin/clavulanic acid, cefoxitin and cefotetan at 70–95%.[29,33,75] For *Porphyromonas* spp., the resistance to penicillin is much lower, ranging between 5% and 10%. The mechanism of resistance is also encoded by the *cfx*A gene.[76] Carbapenems, metronidazole and β-lactam/β-lactamase inhibitor combinations are uniformly active against most isolates of both genera. However, some *Prevotella* isolates have been found that harbour some of the *nim* genes and have a high level of resistance to metronidazole.[61] Clindamycin resistance is rare among *Prevotella* and *Porphyromonas*; if it is detected, it is associated with the presence of either an *erm*F or *erm*G gene.[69-73,75-77] Tetracycline resistance has been reported up to 50% for *Prevotella*[29] (due to the presence of the *tet*(Q), *tet*(M) and *tet*(W) genes) and less frequently for *Porphyromonas* (due to the *tet*(Q) gene).[78] Transfer of tetracycline resistance among *Prevotella* strains has been proven via conjugative transposons, in the same way as observed in *Bacteroides* strains. Tigecycline is highly active against *Prevotella* and *Porphyromonas* strains, with an MIC_{90} ≤1 μg/mL.[71,72] Few studies are available on the activities of clinafloxacin, levofloxacin and moxifloxacin against different species of these genera, but they display good activities against most *Prevotella* and *Porphyromonas* strains, with MIC_{90} values between 0.125 and 2 μg/mL.[29,72]

Among *Fusobacterium* spp., penicillin resistance is still uncommon. There are only a few isolates in which β-lactamase production can be proven by chromogenic testing, but penicillinase has been described in a *Fusobacteruim nucleatum* clinical isolate, and the gene (*fus*1) of a class D β-lactamase has recently been reported.[29,79,80] Amoxicillin/clavulanic acid, piperacillin/tazobactam and carbapenems are highly active against fusobacteria, more than 90% of which are susceptible to cephalosporins, including cefoxitin and cefotetan.[29,33,81,82] Tetracycline resistance has been reported with increasing frequency: both *tet*(W) and *tet*(M) genes have been described among tetracycline-resistant *F. nucleatum* isolates. Clindamycin resistance is observed in 5–10% of the isolates.[33,37,82] Fluoroquinolone resistance is rare among *F. nucleatum* isolates, although resistance levels of up to 10–15% are reported in other fusobacteria.[37,55,72] Metronidazole resistance is uncommon among *Fusobacterium* spp., with the MIC_{90} varying between 0.25 and 1 μg/mL when different species of fusobacteria were evaluated separately.[22] Tigecycline proved very active against *Fusobacterium* isolates tested in recent studies (MIC_{90} = 0.25 μg/mL).[37,55]

Among the less frequently tested Gram-negative anaerobic bacilli, *Bilophila wadsworthia*

often produces β-lactamases, resulting in high MIC_{90} values to penicillin, amoxicillin and piperacillin. However, it is susceptible to clindamycin, cefoxitin, β-lactam/β-lactamase-inhibitor combinations, carbapenems and metronidazole.[29,83] *Campylobacter* (earlier *Bacteroides*) *gracilis*, *Campylobacter rectus* and *Campylobacter* (earlier *Wolinella*) *curvus* vary in their susceptibility to some β-lactams, but remain susceptible to metronidazole, carbapenems and clindamycin. On the other hand, *Sutterella wadsworthensis* (often isolated from the same samples and misidentified as *C. gracilis*) is much more resistant to clindamycin, piperacillin and metronidazole.[84]

5. Resistance Problems Relating to Gram-Positive Anaerobes

5.1 Gram-Positive Cocci

The classification of GPAC has undergone fundamental changes during the past 30 years. *Peptococcus niger* remains the only species in the *Peptococcus* genus, and many other genera have been formed such as *Anaerococcus*, *Finegoldia*, *Micromonas* and *Peptoniphilus*. The genus *Peptostreptococcus* is still a phylogenetically heterogeneous group of bacteria, and is currently undergoing taxonomic revision.[85,86] Because of difficulties in the differentiation of these bacteria in routine laboratories, resistance data are rather scanty and often confused, and referred to as resistance data of GPAC. In general, these organisms demonstrate variable resistance to penicillins (4–14%), clindamycin (4–20%) and metronidazole (0–10%), while retaining much higher or full susceptibility to β-lactam/β-lactamase inhibitor combinations, cephalosporins, carbapenems and chloramphenicol.[31,33,37] Fluoroquinolone resistance among GPAC has been reported to be high, between 14% and 27%.[33,37,75] Higher resistance of GPAC may be observed in certain regions, with rates of 55% for clindamycin[87] and >30% for metronidazole.[88] PBP alterations appear to account for most β-lactam resistance, while the *nim*B gene has been found in a very high proportion of peptostreptococci; nevertheless, it was concluded to be responsible for high-level resistance only in two strains of *F. magna* and silent in 19 of 21 susceptible strains.[89,90] Frequent resistance to tetracycline has been observed, predominantly due to the carriage of the *tet*M gene. In GPAC, constitutive or inducible MLS_B-type resistance may be conferred by *erm*TR, *erm*F or *erm*B elements – *erm*TR being the most predominant element in *F. magna*.[90] The activity of tigecycline was investigated recently during a Europe-wide study for various members of GPAC (*Anaerococcus prevotii*, *F. magna*, *P. micros*, *Peptostreptococcus anaerobius*, and other *Peptostreptococcus* spp.). MIC_{90} values were found uniformly to be very low: 0.12 μg/mL.[71]

5.2 Non-Spore-Forming Gram-Positive Bacilli

Very few data are available concerning the antibacterial susceptibility of non-spore-forming Gram-positive anaerobic bacilli. Most of the genera belonging in this group of bacteria (*Eubacterium*, *Actinomyces*, *Propionibacterium*, *Bifidobacterium* and *Lactobacillus* spp.) are susceptible to β-lactams, including penicillins, cephalosporins, cephamycins, carbapenems, β-lactam/β-lactamase inhibitor combinations and tigecycline.[3,72] Lactobacilli may be resistant to some cephalosporins. *Actinomyces* spp. isolated from different clinical cases may include strains with MICs to tetracycline, moxifloxacin and clindamycin that are higher than the susceptibility breakpoints.[37,91] The antibacterial resistance of *Propionibacterium acnes* has been investigated in Europe-wide studies and the resistance rates to clindamycin, erythromycin and tetracycline have been found to be around 15%, 17% and 3%, respectively.[92,93] The highest resistance occurred among *P. acnes* and *Propionibacterium granulosum* isolated from acne patients and their contacts. In both sets of isolates there were notable inter-country variations, but all strains were susceptible to penicillins, vancomycin, linezolid, ofloxacin and ciprofloxacin.[92,93] Isolates from blood were predominant among the resistant isolates. Most non-spore-forming Gram-positive anaerobic bacilli are resistant to metronidazole. The role of the *nim*A genes in the intrinsically non-susceptible *Propionibacterium* spp.

is unclear; this genus has been suspected of being a possible reservoir of these genes.[62]

5.3 Spore-Forming Gram-Positive Bacilli

Clostridia are largely susceptible to β-lactams, and penicillinase production has only been reported in a few species, such as *Clostridium ramosum*, *Clostridium butyricum* and *Clostridium clostridiforme*.[3] *Clostridium perfringens* is normally susceptible to amoxicillin/clavulanic acid, cefoxitin and imipenem, while *C. difficile* strains are fully susceptible to amoxicillin, but exhibit intrinsic resistance or reduced susceptibility to cephalosporins and imipenem, but not meropenem. With few exceptions, clostridia are susceptible to nitroimidazoles. While some surveys have reported the full susceptibility of *C. difficile* to metronidazole, more and more recent data indicate metronidazole resistance or at least reduced susceptibility to this drug.[94-96] The rate of tetracycline resistance, often encoded by the *tet*M gene in *C. difficile*, may be decreasing, and relatively low values have been reported recently from different countries.[97,98] A recent review summarizes resistance data of *C. difficile* obtained in various geographical areas showing a highly variable differences in the prevalence of resistance to antibacterials other than vancomycin or metronidazole.[99] Tetracycline resistance may be more prevalent (up to about 70%) among *C. perfringens* strains, especially if they originate from veterinary samples.[100] Macrolide and clindamycin resistance is widespread in *C. difficile* strains, varying between 25% and 96%, in contrast with *C. perfringens*, where the resistance rates have remained low.[97-99] Fluoroquinolone resistance has become a major problem among *C. difficile* isolates and is probably the driving force for the spread of some hypervirulent clones, such as ribotype 027.[17,99] The mechanism of fluoroquinolone resistance appears to be associated with *gyr*A and *gyr*B mutations both in *C. difficile* and in *C. perfringens*, with nucleotide substitution found in topoisomerase IV genes in *C. perfringens*.[101,102] At present, rifampicin (rifampin) and rifaximin resistance among clinical isolates of *C. difficile* is rare, but in those strains where the sequence substitution within the RpoB region can be found, the MICs for these drugs are high.[99,103] Co-resistance between rifampicin and three other antibacterials (erythromycin, clindamycin and moxifloxacin) has also been observed.[96] Recent studies from Europe and the US showed a very low MIC_{90} value (0.25 μg/mL) for tigecycline in the case of clinical isolates of *C. difficile*.[71,104] Tests on new antibacterials active against *C. difficile* infections revealed that fidaxomicin (OPT-80) has a low MIC_{90} (0.125 μg/mL),[105] as does REP3123, a novel diaryldiamine that inhibits bacterial methionyl-tRNA synthetases in Gram-positive bacteria.[106]

6. General Considerations Concerning the Treatment of Anaerobic Infections

The two major approaches to the treatment of anaerobic infections involve appropriate antimicrobial therapy and/or surgical management. Surgery is required for the debridement of necrotic tissue, drainage of abscesses, restoration of airspaces, and resection or the maintenance of blood supply, as anaerobic infections regularly cause severe tissue damage or result in abscess formation. Following the development of imaging procedures, these interventions may be performed percutaneously as well.

The antibacterials used for the treatment of anaerobic infections should have activity against both aerobic and anaerobic organisms, as many of these infections involve a mixture of bacteria, mostly originating from the very complex normal flora. Today, antibacterial regimens are usually selected empirically, because the pattern of antibacterial resistance is generally predictable and the testing of clinically relevant anaerobes isolated from a mixed infection is time consuming.

Both the *in vitro* resistance trends observed during the past 20 years in Europe and US among clinically important anaerobes and the correlation with the acquisition of resistance genes enhance concerns about possible treatment failures.[37,38,42] Few studies have definitively associated resistance with clinical failure.[7,23,25,107] Clinical studies concerning the role of anaerobes in the outcome of infections are confounded by

several factors. Most infections are of mixed type, including aerobes and/or facultative anaerobes, and are typically tissue-destructive, requiring debridement. Accordingly, the role of the resistant anaerobe is difficult to determine in this setting. While several retrospective studies have correlated clinical failure with antibacterial resistance,[23,25,107] a prospective observational study of *Bacteroides* bacteraemia clearly demonstrated the association of clinical failure with resistant anaerobes isolated from blood culture.[7]

The impact of resistance trends associated with clinical failures has resulted in recent changes in the recommendations for empirical treatment of some infections involving anaerobes. In cases of intra-abdominal infections, both cefoxitin and cefotetan (used previously most widely in the US) are discouraged as first-line therapy, and clindamycin is no longer listed as first-line therapy either.[108] In Spain, an expert group listed amoxicillin/clavulanic acid as the therapy of choice over penicillin in maxillofacial infections.[109] Unfortunately, a steady increase in resistance against this agent can be observed among different anaerobic species, mostly *Bacteroides* strains.

The widespread presence of determinants conferring resistance to tetracycline and macrolides, carried by conjugative transposons in the *Bacteroides* group strains, is held responsible for the increased resistance to clindamycin as well. Because of this, tetracycline, macrolides and clindamycin are not recommended for the empirical treatment of serious anaerobic infections any more.

The rates of resistance to carbapenems and nitroimidazoles have remained low overall, as has antimicrobial resistance in *Porphyromonas* spp., fusobacteria and, possibly to a somewhat lesser degree, also in *Prevotella* spp. and peptostreptococci. The production of the metallo-β-lactamase coded by the *cfi*A gene is confined to a small subgroup of *B. fragilis*, but the more frequent presence of this gene in clinical and normal flora isolates than the expression of high-level resistance may draw attention to the importance of following the resistance trends in this group of isolates.[47-49] It has been assumed that the producers of this enzyme could be easily selected, not only in the presence of carbapenems but also in the presence of the more commonly prescribed β-lactam/β-lactamase inhibitor combinations. Nevertheless, the absence of increasing rates of carbapenem-resistant isolates should not silence previously expressed concerns about the possibility of a less favourable development in the future.[56]

Several newer fluoroquinolones exhibited good levels of *in vitro* activity against clinically important anaerobic bacteria, and particularly *Bacteroides* spp., when first introduced. However, the rapid selection of quinolone-resistant mutants among anaerobic bacteria with their use or by the use of earlier generation fluoroquinolones may preclude their utility for empirical treatment of serious infections, similarly to aerobes.[66] In targeted treatment, fluoroquinolones can be effective against susceptible isolates.

Tigecycline, the first member of glycylcycline family and derived from a tetracycline nucleus, is a broad-spectrum antibacterial with good antianaerobic activity developed in response to recent increases in antibacterial resistance. Existing and widespread tetracycline resistance mechanisms in anaerobic bacteria have not affected the activity of tigecycline. According to recent clinical studies, the broad spectrum of tigecycline makes it appropriate to use empirically in intra-abdominal and skin and soft tissue infections.[110,111]

Other antibacterials already in clinical practice, in clinical trials or in development were also tested in different recent studies for their activities against anaerobic bacteria. During the testing of telithromycin activity against several anaerobic clinical isolates, contradictory data were observed concerning MIC_{50} and MIC_{90} values. Despite the differences in the *in vitro* circumstances of the testing, which might have been the cause of discrepancies observed in the resistance rates in the different studies, telithromycin may have sufficient activity for therapeutic purposes against certain, but possibly not for all, anaerobic bacteria.[112,113] NVP-LMB415, a novel peptide deformylase inhibitor that targets bacterial metalloproteases, is active against several Gram-positive aerobic bacteria and showed good *in vitro* activity against members of the *Bacteroides* genus as well as several Gram-positive anaerobic bacteria.[114] The antimicrobial

activity of televancin was tested against different Gram-positive and Gram-negative anaerobic bacteria. It had excellent activity against Gram-positive anaerobes (MIC_{90} 2 μg/mL) and was more potent than vancomycin or metronidazole against *C. difficile*. As expected, Gram-negative anaerobic isolates were not inhibited by televancin.[115] The anti-anaerobic activities of three new carbapenems were published recently. Doripenem was as active as imipenem, meropenem and piperacillin/tazobactam, and more active than ertapenem and ampicillin/sulbactam against *B. fragilis* strain. It was even active against isolates that showed resistance to ertapenem, amoxicillin/sulbactam, cefoxitin, clindamycin or moxifloxacin.[116] Sulopenem and the new longer half-life parenteral carbapenem, tomopenem, also have shown equal or better activities as the comparator anti-anaerobic drugs against clinical isolates.[117,118]

New treatment options are needed in the case of severe *C. difficile* infections. Even though vancomycin and metronidazole resistance is rare among the clinical isolates, increased MICs and heteroresistance has been observed by several authors for metronidazole as well as decreased clinical effectiveness without *in vitro* metronidazole resistance of the isolates.[94,119] The suboptimal response in some patients may be related to the vide variety of metronidazole concentrations in watery stools during acute *C. difficile* infection. Even modest increases in the MIC of metronidazole for the clinical isolate may result in insufficient faecal antibacterial concentrations to inhibit *C. difficile*.[99,119,120] In the case of severe and relapsing *C. difficile* infections, non-antibacterial-based therapies have also been evaluated, including the use of a toxin-binding agent (tolevamer); however, it failed in phase III clinical trials in the US and also in Europe.[121,122] Immune agents (toxoid vaccine, hyperimmune globulin) are also being considered for prevention and treatment of *C. difficile* infections.[123]

7. Conclusions

Antimicrobial resistance among anaerobes continues to rise, which is generally not surprising as there have been parallel observations among aerobes over the last several decades. Awareness of this problem through more surveillance testing in different parts of the world involving more anaerobic bacteria belonging to different genera, using standardized methods, will help to more successfully guide empirical antibacterial treatment in anaerobic infections.[124] Striking geographical and hospital-specific differences in susceptibility patterns, related very probably to different antibacterial prescribing habits, emphasize the need for more individual isolate testing in the case of severe infections involving anaerobes, which can directly affect antibacterial choice and will hopefully result in better patient outcomes.

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