Infections Caused by Anaerobic Microorganisms

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Introduction

Anaerobic bacteria are the most numerous components of the normal human microbiota colonizing the mucous membranes. They are dominant commensals in mucosal surfaces such as the oral cavity and the gastrointestinal and female genital tracts. In some circumstances, especially after the breakdown of mucosal barriers, these microorganisms may spread from indigenous microbiota into normally sterile body sites and they can be responsible for severe disease like in blood infections. The presence of anaerobic microorganisms predominates in several clinical syndromes, and this fact could be attributed to the large numbers of these bacteria residing on mucous membranes, the production of a wide variety of virulence factors, the synergy with other aerobic bacteria and the increased resistance of these microorganisms to some antimicrobials. However, most clinically significant anaerobes are involved in mixed infections alongside aerobic bacteria.

Infections due to anaerobic isolates may sometimes be missed because of the special measures required for their transportation. Other critical factors for the successful isolation of these microorganisms in the microbiology laboratory include incubation in anaerobic atmosphere, the use of specialized culture media, and prolonged culture (Finegold, 1995). Moreover, several infections caused by anaerobes are important due to the fact of the observation of higher resistance rates of these microorganisms to some antimicrobial agents over the last years (Brook et al., 2013). The combination of all those factors makes it crucial to know when an anaerobic infection is vital in order to use appropriate microbiologic methods to identify the bacteria and to select the correct treatment.

This chapter focuses on the description of the main genera of anaerobic bacteria that causes human infections and their main syndromes in which they are implicated (Table 1).

Table 1 Main anaerobic bacteria and clinical syndromes in human population.

Type of bacteria	*Microorganism*	*Infectious syndrome*
Gram-negative bacilli	*Bacteroides* spp.	Abdominal infections, peritonitis, bacteremia, genital infections, abscess
	Porphyromonas spp.	Orofacial infections
	Prevotella spp.	Orofacial infections, periodontitis, abdominal infections, genital infections, bacteremia
	Fusobacterium spp.	Cerebral abscess, bacteremia, orofacial infections, pneumonia, Lemière syndrome
Gram-positive bacilli	*Clostridium* spp.	Bacteremia, skin and soft-tissue infections, diarrhea (colitis pseudomembranous), necrotizing enterocolitis, tetanus, botulism
	Actinomyces spp.	Cerebral abscess, mastoiditis, genital infections, neck and head infections, lung infections
	Cutibacterium acnes	Medical devices infections
	Bifidobacterium spp.	Cervical lymphadenitis, abdominal infections, periodontitis
	Eubacterium spp.	Periodontitis, odontogenic abscess, bite infections
Gram-positive cocci	*Finegoldia magna*, *Parvimonas micra*, *Peptoniphilus* spp., *Anaerococcus* spp., *Peptostreptococcus* spp.	Skin and soft-tissue infections, abdominal infections, abscess, bacteremia
Gram-negative cocci	*Veillonella* spp.	Polymicrobial infections, bacteremia

Anaerobic bacteria present in the human microbiota

Human microbiota is integrated by different kind of microorganisms of which anaerobic bacteria are the most frequent of them (Huttenhower et al., 2012). Until now, culture of specimens has been the gold standard of microbiology for the characterization of microorganisms that make up the microbiome. However, many of the bacteria isolated from these samples were no cultivable, as it was demonstrated in a study (Gill et al., 2006). Today, new techniques based on DNA analysis are being used in order to characterize new pathogens as well as anaerobic bacteria. Anaerobes are especially present in mucosal surfaces like the gastrointestinal and female genital tract and the oral cavity. The asymptomatic carriage of *Clostridioides difficile* has been confirmed in the human gastrointestinal tract in a small number of healthy people. The number of colonized persons increases during hospitalization and among those who are taking antimicrobial agents. The microbial species and concentrations varies depending on the location; the highest anaerobes concentration is found in the oral mucosa with concentrations ranging 10^2/mL to 10^{12}/mL. In the gingival area, the anaerobe:aerobe ratio is1000:1. The main anaerobic microorganisms present in this location are species of the genera *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Bacteroides*.

Regarding to the microbiota of the stomach and upper intestine, low number of anaerobic bacteria are present in this area; in persons with decreased gastric acidity, the microbiota of the stomach is similar to those present in the oral cavity. However, the microbiota of the upper intestine resembles those present in the colon. The predominant anaerobes present in this last location are species of *Bacteroides* spp., *Clostridium*, *Peptostreptococcus* and *Fusobacterium*.

Regarding to the normal female genital tract, the ratio anaerobic:aerobic range of 1:1 to 10:1 The predominant anaerobic species are *Prevotella*, *Bacteroides*, *Fusobacterium*, *Clostridium* and *Lactobacillus*. *Bacteroides* spp. is found in the genital tract of approximately 50% of women and supposes a 15% of the microbial population.

Anaerobic microorganisms also may be present in areas that are exposed to air; the skin microbiota contains species of anaerobes such as *Cutibacterium* (formerly *Propionibacterium*) *acnes*, other species of *Cutibacterium* and *Peptostreptococcus*.

Fig. 1 shows macroscopic aspect of some anaerobic microorganisms.

Taxonomic and microbiologic classification

Despite the great number of anaerobes present in the normal human microbiota, only relatively few species are involved in human infections. A significant fact is that infections involving anaerobic pathogens are often polymicrobial and usually are produced as a consequence of the disruption of mucosal surfaces by surgery, trauma, tumors or ischemia and the spread of microbiota to sterile sites.

Anaerobic microorganisms are divided in Gram-negative and Gram-positive and in bacilli and cocci. There are also some Gram variable microorganisms such as *Actinomyces* spp., *Clostridium tetani* and *Cutibacterium acnes*, alternating between a Gram-negative and Gram-positive phase depending on the replication-stationary stage (Beveridge, 1990).

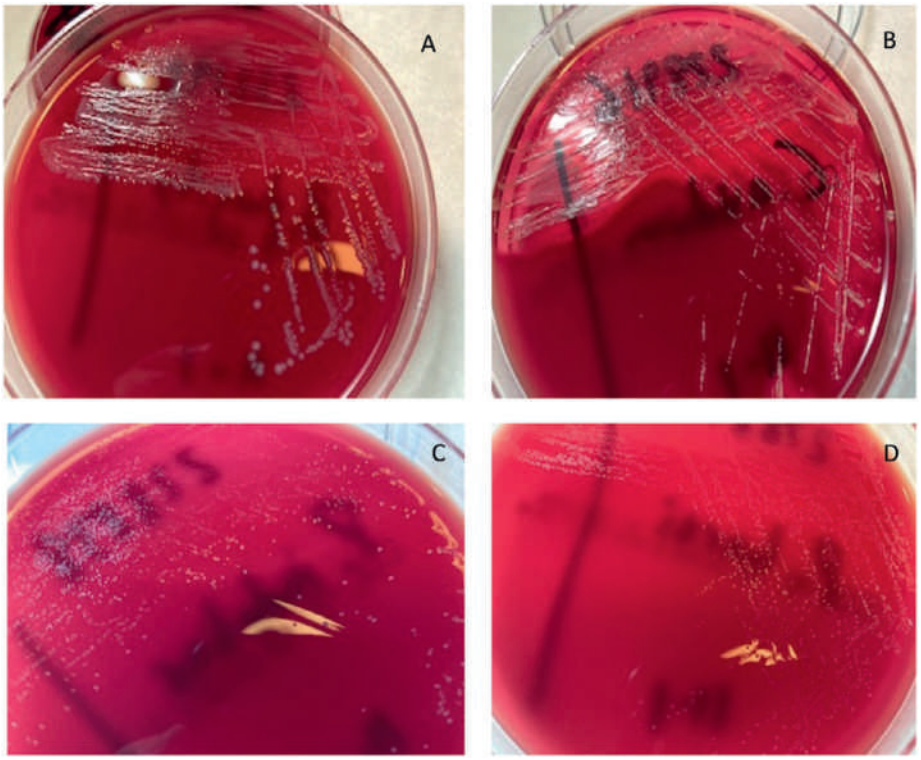

Fig. 1 Microphotographs showing the macroscopic aspect of some anaerobic microorganisms isolated in blood agar: (A) *Bacteroides fragilis*. (B) *Cutibacterium acnes*. (C) *Peptoniphilus harei*. (D) *Finegoldia magna*.

Gram-negative anaerobic bacilli

Bacteroides, *Prevotella*, *Porphyromonas* and *Fusobacterium* species are the most frequent anaerobes in this group isolated in clinical samples and involved in human infections, especially the *B. fragilis* group (Wexler, 2007). Of this group, *B. fragilis* is the species most often isolated from human infections. Other members of this group include *B. thetaiotaomicron*, *B. ovatus*, *B. vulgatus* and *B. distasonis*. A definitive taxonomic classification of this genus was performed based on the study of 16S rRNA (Paster et al., 1994). These are microorganisms commonly involved in intraabdominal infections and other conditions such as decubitus ulcer and diabetic foot ulcers. Moreover, the disruption of the mucosal barrier becomes in abscess formation and bacteremia. *B. fragilis* virulence is mostly by a toxin production. Enterotoxigenic *B. fragilis* strains secrete a termed enterotoxin and may have a role in inflammatory diarrhea and flare-ups of inflammatory bowel disease (Sears, 2009; Basset et al., 2004).

Prevotella (both pigmented and non-pigmented species) and *Porphyromonas* species are anaerobic Gram-negative bacilli involved in oral cavity infections and also they are the most often anaerobes isolated from respiratory infections and their complications. As a consequence of their presence in infections of the oral cavity, these microorganisms can spread within the intracranial cavity and could produce brain abscesses.

Finally, regarding to *Fusobacterium* species they are mainly part of the microbiota of the oral cavity and the intestinal tract and they are able to produce abscesses and necrotizing infections such as pneumonia and brain infections.

Gram-positive anaerobic bacilli

Among the anaerobic Gram-positive bacilli, *Clostridium* spp. is most commonly isolated from human infections. These microorganisms are usually associated with wound and bloodstream infections and abscesses. There is more than 200 species of *Clostridium*, but the most common species isolated from clinical samples are *C. perfringens*, *C. septicum*, *C. ramnosum*, *C. novyi* and *C. sordellii* (Brook, 2016). *C. difficile* may cause several intestinal diseases that range from mild sickness to severe intestinal infections called pseudomembranous colitis.

Regarding to the anaerobic Gram-positive non-spore-forming rods, these bacteria are part of the microbiota of different body sites like vagina, skin, gingival crevices and gastrointestinal tract. This group include *Cutibacterium* (formerly *Propionibacterium*), *Bifidobacterium*, *Lactobacillus*, *Actinomyces*, *Eubacterium*, *Atopobium* and others. These anaerobes may be isolated from aspiration pneumonia, peritonitis, periodontitis, gynecological infections and intracranial abscesses. *C. acnes* is the most frequent species included in genera *Cutibacterium* and usually has low virulence, but it might cause infections in implanted prostheses and medical devices as well as in central nervous system (CNS) shunts and drains.

Gram-positive anaerobic cocci (GPACs)

Among the GPAC associated with clinical infections, the most prominent isolated bacteria from human samples are *Anaerococcus*, *Finegoldia*, *Parvimonas*, *Peptoniphilus* and *Peptostreptococcus*. GPACs are mostly isolated from polymicrobial infections so their relevance has often been overlooked. Moreover the taxonomy of GPACs has undergone various changes over the years, mainly due to the introduction of new molecular identification methods. These anaerobic bacteria are part of the oral, upper respiratory, genitourinary and intestinal tract and skin microbiota and are isolated from infections such as lung abscesses, aspiration pneumonia, soft tissue infections, female genital tract and chronic sinusitis.

Gram-negative anaerobic cocci

From the three described genera of Gram-negative anaerobic cocci, *Veillonella*, *Acidaminococcus* and *Megasphaera*, the first of them is that it has epidemiological relevance. Its clinical meaning is unknown and they are considered microorganisms of low virulence. The most relevant species are *V. parvula*, *V. atypica* and *V. dispar*, which are part of the microbiota of the oral cavity and the intestinal and genitourinary tract. They are mainly isolated within polymicrobial infections, although in some circumstances such as malignancies may cause bacteremia (Cobo et al., 2020a, b).

Pathogenesis and virulence factors of anaerobic bacteria

Once anaerobic microorganisms are present in tissue with a lowered oxidation-reduction potential, they may proliferate and cause local infection. After this, there are some factors involved in the pathogenesis of these kinds of infections such as bacterial synergy, virulence factors of the bacteria, and mechanism of abscess formation. Some of these virulence factors are showed in Table 2. Virulence factors may contribute to anaerobic infection due to its ability to evade host defenses, produce enzymes and toxins, adhere to cell surfaces and exhibit some surface structures like capsular polysaccharides.

Regarding to the GPACs, in two of them has been possible to identify some virulence factors. The well-described virulence factors of *F. magna* are Protein L (Björck and Protein, 1988), subtilase (SufA) (Karlsson et al., 2007), peptostreptococcal albumin binding protein (PAB) (De Château and Björck, 1994) and *F. magna* adhesion factor (FAF) (Frick et al., 2008).

Protein L induces an effect on human basophils inducing the synthesis of leukotriene C_4 (LTC4) (Patella et al., 1990). LTC4 is a proinflammatory mediator that induces a greater histamine release and the synthesis of the prostaglandin D_2. These two mediators have significant importance in inflammation, so expression of protein L is correlated with virulence (Kastern et al., 1990).

Table 2 Main virulence factors of anaerobes.

Microorganism	*Virulence factor*
Finegoldia magna	Protein L
	SufA (subtilase)
	PAB (peptostreptococcal albumin binding protein)
	FAF (*F. magna* adhesion factor)
Parvimonas micra	Capsule formation
	Adhesins
	Immunoglobulin Fc-binding proteins
	Gelatinase and protease activity
	Production of TNF-α and IL-1β
Bacteroides fragilis group	Hemagglutinin
	Protease and neuraminidase activity
	Capsular polysaccharides
	Enterotoxin production
Prevotella species	Protease production
	Lipopolysaccharides
Fusobacterium species	Proteases production
	Lipopolysaccharides
	Leukotoxin production
	Hemolysin and phospholipase (*F. necrophorum*)
	Adhesins (*F. nucleatum*)
Porphyromonas gingivalis	Capsule
	Hemolysin
	Proteases
	Lipopolysaccharides

In addition, protein L is a potent inducer of the synthesis and release of IL-4 and IL-13 and classifies this protein as a bacterial super-antigen (Genovese et al., 2003).

Protein PAB is a human serum albumin present in several isolates of *F. magna* whose presence suggests a link between this phenotype and the presence of suppurative infections. Therefore, protein PAB plays an important role in enhancing bacterial virulence during infection.

On the other hand, subtilisin-like proteinase of *F. magna* whose presence may result in enhanced pathogenicity of this anaerobe during the infection. The ability of SufA to cleave and inactivate some antimicrobial peptides might lead to increase survival and proliferation during infection. Moreover, several studied showed that SufA was able to modulate the activity of chemokines to promote bacterial survival during epithelial inflammation decreasing the inflammatory response (Karlsson et al., 2009a). In addition, the role of SufA in the interaction with the host coagulation system was also studied. SufA was found to cleave fibrinogen which affects to fibrin polymerization which prevent the formation of a fibrin network around *F. magna* and to the wound healing that leads to the delay of wound repair (Karlsson et al., 2009b). The final effect is that the bacteria are trapped and this fact facilitates the establishment of infection and the promotion of virulence (Karlsson et al., 2009a).

Finally, the FAF protein is a factor that mediates bacterial aggregation. The presence of this protein in the *F. magna* surface could impair wound healing in chronic wounds. Moreover, this factor was found to have a neutralizing capacity on bacterial proteins such as LL-37, MK and hBD-3 (Frick et al., 2011).

Regarding to *Parvimonas micra*, this is one of the best studied microorganisms belonging to the GPAC group in terms of characterization of its virulence factors. The capacity of capsule formation and the ability to form hydrogen sulfide have been demonstrated to be as important virulence factors (Carlsson et al., 1993). Moreover, *P. micra* have the capacity to adhere to gingival epithelial cells (Dzink et al., 1989) and to express immunoglobulin Fc-binding proteins (Grenier and Michaud, 1994). In addition, some data in several strains of *P. micra* demonstrated to have cell-associated gelatinase activity (Ng et al., 1998) and elastase activity (Mikamo et al., 1999). Finally, some studies also reported the *P. micra* production of TNF-α and IL-1β which are important determinants of the periodontitis progression (Tanabe et al., 2007).

In Gram-negative anaerobic microorganisms, some virulence factors have been also identified. Regarding to *B. fragilis*, this microorganism has a high ability for abscess formation. *Bacteroides* species have involvement in the prolongation of the intrinsic pathway of clotting in human blood (Murphy et al., 2011). *B. fragilis* and other *Bacteroides* species such as *B. thetaiotaomicron* cause the release of significant levels of bradykinin in human plasma that will provide the bacteria the ability to spread by inhibition of clot formation (Murphy et al., 2011). However, the capsular polysaccharide has been identified as the major virulence factor of *B. fragilis*, playing a central role in the abscess production (Onderdonk et al., 1977). *B. fragilis* may express different surface polysaccharide combinations through a reversible inversion of DNA segments (Krinos et al., 2001). The most important and most studied of these polysaccharides are PSA and PSB; PSA was demonstrated to activate host $CD4^+$ T cells and facilitate the release of interleukin-17, interferon-γ, and chemokines (Chung et al., 2003; Wang et al., 2006). Moreover, the capsule promotes the release of the proinflammatory cytokines tumor necrosis factor-α and IL-1β that are found to potentiate the increase of cell adhesion molecules in order to increase the binding o neutrophils to these cells and initiates abscess formation (Gibson et al., 1998).

On the other hand, *B. fragilis* produces other virulence factors such as fimbriae, pili and hemagglutinin molecules that help in attachment to host cell surfaces. Moreover, they produce several toxins and enzymes like neuraminidase, protease, hydrolase and superoxide dismutase. Enterotoxigenic *B. fragilis* strains secrete an enterotoxin termed *B. fragilis* toxin (BFT) and may have a role in inflammatory diarrhea and flare-ups of inflammatory bowel disease (Basset et al., 2004; Sears, 2009). This toxin has a cytopathic effect for epithelial intestinal cells inducing tissue damage.

On the other hand, *C. difficile* produces two toxins, an enterotoxin (toxin A) and a cytotoxin (toxin B). In some strains a third toxin (binary toxin) could be produced, although the importance in the pathogenesis of this disease has not been clearly established. Apparently, toxins A and B are able to interact synergistically in the pathogenesis of *C. difficile* infection, although toxin-A negative strains can also produce disease. Highly virulent *C. difficile* (BI/NAP1/PCR ribotype 027) strains have caused some outbreaks. These isolates are characterized by high level toxin production due to a deletion in the negative regulator gene (tcdC) of toxin A and B. Other *Clostridium* species produce some toxins such as neurotoxins, enterotoxins, lecitinases, DNAses and others and this fact justify their virulence and involvement in aggressive clinical pictures.

F. necrophorum and *F. nucleatum* produce a numerous types of toxins such as leukotoxin, endotoxin and hemolysin causing several necrotic diseases and oral infections. *F. nucleatum* also co-aggregates with other oral bacteria to increase attachment to plaque helped with some adhesin molecules. Both species of *Fusobacterium* produce a lipopolysaccharide responsible for the production of cytokines and other inflammatory mediators playing a central role in periodontal disease.

Regarding to *Prevotella* species little is known about the virulence factors, although production of proteases and metabolic products has been described, especially IgA proteases. The destruction of Ig A produced by mucosal surfaces permit this anaerobe to evade the first line of defense.

The last Gram-negative anaerobe which is known producing virulence factors is *P. gingivalis*. This bacterium produces proteases that can help to cause attachment, degradation or cleavage of host cell proteins and surface receptors, adhesins and hemagglutinins. The capsular polysaccharide of this microorganism may facilitate the spread of infection because it has a proinflammatory activity implicated in the periodontal disease.

Clinical syndromes associated with anaerobic microorganisms

Anaerobic bacteria are common pathogens in humans and they can produce a great variety of infections in different body locations. Table 3 summarizes the type and location of these infections. Although most clinically significant anaerobes are involved in mixed infections alongside aerobic bacteria, they can be responsible for severe disease in certain circumstances, such as in blood infections or when present in normally sterile body sites. Infections due to anaerobic isolates may sometimes be missed because of the special measures required for their transportation. Other critical factors for the successful isolation of these microorganisms in the microbiology laboratory include incubation in anaerobic atmosphere, the use of specialized culture media, and prolonged culture (Finegold, 1995). The main feature of infection caused by anaerobes is the abscess formation, both in the site of direct bacterial contamination and in distant sites due to contiguous and hematogenous spread.

Central nervous system infections

In this region, the main types of infections in which anaerobic bacteria are involved include subdural empyema and both brain and epidural abscesses. Meningitis caused by anaerobes is quite rare and often suggests a parameningeal collection or shunt infection. However, anaerobic microorganisms are common pathogens producers of brain abscesses (Brook, 2017; Le Moal et al., 2003). This clinical entity is caused by microorganisms belonging to the normal oral cavity microbiota as a consequence of its spread from otitis, sinusitis or oral infections. On the other hand, it is not unusual the hematogenous dissemination from intraabdominal or pelvic infections, leading to the production of infections located at the brain region. In these last circumstances, the microorganisms implicated are those present in the intestinal and/or female genital tract microbiota. The microbiology of these abscesses may include a great variety of pathogens; it may present as an isolated infection by anaerobes or a mixture of aerobic and anaerobic bacteria, being the anaerobic bacteria common constituents of brain abscesses. The main anaerobes implicated in the production of brain abscesses are GPACs, *Prevotella*, *C. acnes*, *Fusobacterium*, *Bacteroides*, and *Actinomyces* (Brook, 2017).

Table 3 Types and location of main infections caused by anaerobic microorganisms.

Location of infection	*Infection*
Central nervous system	Epidural abscess
	Subdural empyema
	Brain abscess
Head, neck and oral cavity	Sinusitis
	Gingivitis
	Dental infections
	Periodontal infections
	Perimandibular infection
Chest cavity	Pneumonia
	Pneumonitis (aspiration)
	Pulmonary abscess
	Empyema
Abdominal cavity	Intraabdominal abscess
	Liver abscess
	Biliary tract infection
	Peritonitis
	Appendicitis
Female genital tract	Pelvic inflammatory disease
	Pelvic abscess
	Bartholin gland abscess
	Endometritis
	Bacterial vaginosis
	Post-surgery infection
Blood	Bacteremia
Skin and soft tissue	Cutaneous abscess and wound
	Diabetic foot ulcer
	Pressure ulcer
	Gas gangrene
Bone and joints	Arthritis and osteomyelitis

Head, neck and oral cavity infections

Anaerobic microorganisms are involved in some infections of the oral cavity and adjacent tissues. The bacteria isolated from these infections reflect the microorganisms present in the normal oral microbiota, especially *Bacteroides* non-*fragilis*, pigmented *Prevotella* species, *Porphyromonas*, *Fusobacteria* and *Peptostreptococcus*.

Dental infections

Dental infections include a group of diseases such as periapical or dental abscesses, pulpitis and perimandibular space infections and anaerobes are involved in most clinically important infections in this area. The initial lesion is endodontal and the infection progress to the periapical region and it may spread through the mandible to involve adjacent tissues causing osteomyelitis of the maxillary sinuses and infection of submandibular spaces. Factors involved in this progression are unknown but the presence of gingival microbiome forming a "network of bacteria" may contribute (Siqueira and Rôças, 2013).

Also, in the gingival crevices and gums, anaerobic microorganisms are able to cause gingivitis, periodontitis and periodontal abscesses. Formation of dental plaque influenced by several factors like oral hygiene, leads to overgrowth of pathogenic bacteria and development of infections in this location. The bacteria mainly involved in these kinds of infections are *Prevotella*, *Fusobacterium*, *Porphyromonas* and *Treponema* spp.

The most common type of periodontitis is chronic periodontitis, a major cause of tooth loss. However, gingivitis may become a more serious infection known as Vincent angina or trench mouth. This disease is a necrotizing ulcerative process associated with severe pain, tender bleeding gums, tissue destruction, pseudomembrane formation and putrid discharge. Patients may have fever, cervical lymphadenopathy and leukocytosis. This infection may spread to adjacent tissues causing bone or soft tissue destruction or acute necrosis of the pharynx.

Another necrotizing infection of the oral mucous membranes is noma or cancrum oris. As in the Vincent angina, this infection is characterized by destruction of bone and soft tissue. In the most severe cases, it can evolve quickly to from gingival inflammation to orofacial gangrene (Enwonwu et al., 2006). The majority of cases are encountered in sub-Saharan Africa in young children with malnutrition or systemic disease (Falkler et al., 1999).

Deep neck space infections

The deep neck spaces are formed by fascial planes of the head and neck such as the submandibular space. The infections in these areas often emerge from dental infections rather than from pharynx or tonsils infections.

There is two severe and life-threatening perimandibular infections known as Lemierre syndrome and Ludwig's angina.

Ludwig's angina consist of a bilateral infection of the sublingual and submandibular spaces accompanied by marked local tissue swelling (base of the tongue), tongue displacement and potential airway compromise. This disease is typically a polymicrobial infection involving the oral cavity microbiota.

On the other hand, Lemierre's syndrome is an infection of the posterior compartment of the lateral pharyngeal space with subsequent septic thrombophlebitis of the internal jugular vein, bacteremia and lung metastatic abscesses. This entity is usually caused by *F. necrophorum* but other anaerobic microorganisms such as *Bacteroides*, *Peptostreptococcus*, *Porphyromonas* and *Prevotella* have been involved in this infection (Kuppalli et al., 2012; Klug et al., 2016).

Other infections

Although rare, anaerobic microorganisms are able to produce chronic sinusitis in both adults and children. The predominant anaerobes involved in these infections are *Fusobacterium*, *Peptostreptococcus*, *Prevotella* and *C. acnes*. However, the majority of these infections are caused by mixed infections of aerobic and anaerobic bacteria (Brook, 2008a).

Chest cavity infections

Anaerobic bacteria are relatively common pathogens isolated from infections of the pleuropulmonary area. These infections are predominantly acquired due to aspiration of oral or dental secretions by patients with predisposing conditions such as neurologic disorders, periodontal or gingival disease and/or situations with temporary loss of consciousness (Bartlett, 2012).

The anaerobic microorganisms most commonly associated with pleuropulmonary infections are those that form part of the oral cavity and include *Prevotella*, *Peptostreptococcus*, *Bacteroides* and *Fusobacteria*. However, a great quantity of these infections are mixed anaerobic and aerobic, especially microaerophilic streptococci (Takayanagi et al., 2010; El-Sohl et al., 2003).

Clinical presentation may occur in four ways: aspiration pneumonitis, necrotizing pneumonia, lung abscess or empyema. Aspiration pneumonitis is generally an indolent form of pneumonia in contrast to the abrupt course of acute pneumonia. Patients often present with symptoms of chronic disease, including anemia and weight loss. Initially, patients rarely have putrid sputum although can become malodorous in the later stages of disease. It can see a mixed microbiota in the Gram stain and the infection is produced in a dependent pulmonary segment (Bartlett, 2012). Samples obtained by transtracheal and/or transthoracic aspiration may be adequate for the diagnosis.

On the other hand, necrotizing pneumonia is a complication of the aspiration pneumonitis and is characterized by the presence of many abscesses within the lung parenchyma.

Regarding to the lung abscesses, the majority of them are secondary to the presence of periodontal disease and in these cases the predominant bacteria are those present in the oral microbiota. Finally, empyemas are long-term complications of anaerobic pulmonary infection and are characterized by pleuritic chest pain.

Abdominal cavity infections

The main intraabdominal infections caused by anaerobic microorganisms are the peritonitis, both generalized and localized, and the abscesses. These entities are usually polymicrobial and mixed infections (aerobic and anaerobic bacteria), with a predominance of coliforms, anaerobes and *Streptococcus*/*Enterococcus*. They accounted due to a break in continuity of the mucosal surface and the leak of the normal intestinal microbiota into the sterile peritoneal cavity. The main causes of the mucosal break are diseases such as neoplasms, diverticulitis, appendicitis, inflammatory bowel disease, surgery or trauma. The majority of bacteria isolated in these kinds of infections are *Escherichia coli* and *Bacteroides* spp., especially *B. fragilis* group. Other anaerobic microorganisms commonly isolated are *Prevotella*, *Peptostreptococcus* and *Fusobacterium* spp. Regarding to the proximal bowel infections, the perforation of this area is followed of infection due to aerobic and anaerobic Gram-positive bacteria and *Candida* spp. Moreover, infections in this region involve other microorganisms like *C. septicum* and other clostridia.

The toxin-producing *C. difficile* is responsible for antibiotic-associated gastrointestinal diseases ranging from a relatively benign, self-limited diarrhea to a severe, life-threatening pseudomembraneous colitis. The antibiotics alter the normal microbiota allowing the overgrowth of these relatively resistant microorganisms. The disease is produced due to the proliferation of this bacterium in the colonic mucosa and the production of toxins.

Female genital tract infections

The female genital tract is a body region that constitutes a major reservoir for anaerobic microorganisms. The majority of infections of the female genital tract that are not caused by sexually transmitted agents involve anaerobes. The main infections that can be considered within this section are endometritis, Bartholin gland abscesses, bacterial vaginosis, salpingitis, pelvic cellulitis, septic thrombophlebitis, wound infections, tubo-ovarian abscesses, pyometra, adnexal abscesses and pelvic inflammatory disease, and septic abortion. The main bacteria implicated in these kinds of infections include *Prevotella*, *Porphyromonas*, *Peptostreptococcus*, *Actinomyces*, *Eubacterium* and *Clostridium*. Like in the majority of anaerobic infections, most cases are produced by mixed infections, involving both aerobes and anaerobes (Cereija et al., 2013).

Anaerobic bacteremia

Anaerobic microorganisms remain an important cause of bloodstream infection being still a major cause of morbidity and, despite all the advances in medical practice in recent years, the presence of anaerobes in the bloodstream continues to have a high associated mortality requiring appropriate treatment (Raymond et al., 2006; Blairon et al., 2006). The detection rate of anaerobes in blood cultures is around 0.5–11.8% of all bacteremic episodes, depending on geographic location, and both patient age and condition (Arzese et al., 1995). Mortality from anaerobic bacteremia has remained high over the past few years. Mortality rates reported range from 14% (De Keukeleire et al., 2016) to 27% (Robert et al., 2008). In a very recent report, the mortality rate was 25.7%, similar to other investigations (Cobo et al., 2020a, b). A study obtained a mortality rate of 14% for patients with adequate antimicrobial therapy compared with 63% for patients with inappropriate treatment (Zahar et al., 2005).

Some factors frequently associated with high mortality from anaerobic bacteremia have been published including underlying malignancies, especially hematologic, diabetes and gastrointestinal surgery (Blairon et al., 2006; Lassmann et al., 2007). A below mentioned report (Cobo et al., 2020a, b), moreover, found three variables significantly and independently associated with mortality with anaerobic bacteremia: hospitalization in an ICU, presence of septic shock and presence of any type of cancer.

Most anaerobic bacteriemias are caused by Gram-negative bacilli, especially by members of *B. fragilis* group (60% cases), followed by *Clostridium*, *Peptostreptococcus* and *Fusobacterium* species (Brook, 2010). Some of these bacteriemias could be polymicrobials (Cobo et al., 2018).

Anaerobic bacteriemia is often secondary to an infectious process derivate form an intraabdominal, female genital tract, respiratory tract or soft tissue source. Predisposing factors for this infection are mainly debilitating diseases such as malignancies, diabetes, organ transplantation and abdominal and pelvic surgeries (Brook, 2010).

Skin and soft tissue infections

Infections in the skin and soft tissue caused by anaerobic microorganisms are mainly due by contamination with the flora from adjacent mucosal surfaces, although can be also caused by cutaneous anaerobic microbiota, especially *Peptostreptococcus* (Brook, 2007). The main clinical entities in this type of infections are cutaneous abscess and wounds, diabetic foot ulcers, pressure ulcers and gas gangrene. As in other areas, infections most often are polymicrobials, yielded mixed microbiota, including aerobes and anaerobes, mainly the *B. fragilis* group and *Clostridium* spp. along with Enterobacteriaceae and *Enterococcus* spp. However, in infections caused near to oropharynx region, predominant pathogens are those present in the oral microbiota such as *Porphyromonas*, *Prevotella*, *Fusobacterium* and *Peptostreptococcus* spp. Anaerobic microorganisms can also cause deep soft tissue infections like

synergistic cellulitis, necrotizing fasciitis and gas gangrene; these infections are usually mixed infections with aerobes and anaerobes. The most important pathogens involved in deep infections are *Clostridium*, *Bacteroides*, *Peptostreptococcus* and group A β-hemolytic streptococci. A subtype of cellulitis such as Fournier gangrene may require also surgical treatment and it is characterized for affectation of perineum, scrotum and the anterior abdominal wall.

Bone and joint infections

Arthritis is rarely caused by anaerobic microorganisms and most cases involve a single isolate and are secondary to hematogenous spread, trauma, or a prosthetic joint. The most frequent anaerobes encountered in this kind of infection are *B. fragilis* group, *Fusobacterium*, and *Peptostreptococcus* spp. (Brook, 2008b). In infections involving prosthetic devices, especially in shoulder joints, Cutibacterium could be sometimes isolated these types of samples (Levy et al., 2008).

Regarding to osteomyelitis, the predominant anaerobes in this disease are *Bacteroides* spp., while *Prevotella*, *Porphyromonas*, *Peptostreptococcus* and *Fusobacterium* are the predominant anaerobes in skull and bite infections. On the other hand, in osteomyelitis associated with wound contamination after trauma or exposure to gut microbiota, *Clostridium* spp. may be also isolated.

The main sources encountered in osteomyelitis infections are infected adjacent soft tissue areas, such as foot and decubitus ulcers, in which is frequent the isolation of aerobes and anaerobes.

Laboratory diagnostics of anaerobic infections

Samples collection and transportation

The samples should be properly collected and transported. Samples should be collected minimizing contamination by indigenous microbiota of mucosal surfaces and it is also important to remember that the samples should be taken, if it is possible, before starting antimicrobial therapy. The optimal samples are usually sterile fluids such as blood, pleural and peritoneal fluids, and also biopsy specimens. Table 4 describes the main acceptable collection procedures for the recovery of anaerobes. As it can see, in general, collection of tissues or sterile fluids is better than swab samples. The percutaneous aspiration from an abscess requires decontamination of the skin or mucosal surface. The sample should be immediately inoculated in a commercially available anaerobic-transport medium or, as alternative, to inoculate the sample in a sterile tube and cover up to the surface eliminating or minimizing the quantity of air. After the collection, a rapid transportation into the microbiology laboratory is necessary in order to preserve the anaerobes and to permit prompt microbiologic processing, because a contact with O_2 makes unviable the anaerobic microorganisms in less than 1 h.

Direct examination of the sample and gram staining

Direct macroscopic examination of the specimens for study of anaerobes may reveal several features that cause suspicion of anaerobic infection such as foul odor (due to the presence of volatile fatty acid and amine-end products of metabolism), red fluorescence due to pigmented *Prevotella* or *Porphyromonas* species, and black necrotic tissue, discharge, gas or purulence. However, the absence of these characteristics does not rule out anaerobic infection.

On the other hand, microscopic examination of the sample should include a Gram stain that may reveal the number and morphology of microorganisms and the presence of neutrophils. Because anaerobic infections are usually polymicrobial, Gram stain of exudates showing a polymicrobial microbiota could be indicative of anaerobic infections. Some anaerobes have special characteristics when they are observed by microscopy: *F. nucleatum* is a fusiform bacterium with pointed ends, *F. necrophorum* is a long "ropy" Gram-negative bacillus and *Clostridium* spp. are large "boxcar"-like Gram-positive bacillus. In some circumstances, the direct microscopic examination can lead to final diagnosis like in case of bacterial vaginosis due to the presence of "clue" cells. The presence of branching Gram-positive bacilli can mean the presence of *Actinomyces* spp., *Propionibacterium* spp. or *Bifidobacterium* spp.

Table 4 Acceptable collection procedures for anaerobic microorganism culture.

Blood collection for culture
Aspiration of sterile fluids (pleural, peritoneal, . . .)
Suprapubic puncture for urine collection
Percutaneous tracheal aspiration
Bronchoalveolar lavage
Transthoracic pulmonary puncture
Abscess percutaneous aspiration
Deep aspiration of cutaneous ulcer
Transvaginal aspiration
Biopsy samples

Sample processing: Anaerobic culture techniques

Selective and nonselective media should be used for culture of samples. As a nonselective medium, it can use the anaerobic *Brucella* blood agar (containing horse or sheep blood, additional hemin, and vitamin K_1). For the selective isolation of the *Bacteroides fragilis* group and *Bilophila*, *Bacteroides* bile-esculin agar could be used; for the selective isolation of pigmented and non-pigmented *Prevotella* and other Gram negative anaerobic rods, kanamycin-vancomycin agar with laked sheep blood to select could also be used; other media such as phenylethyl alcohol sheep blood agar may be used to inhibit facultative Gram negative rods. Finally, to inhibit swarming of several clostridia, an anaerobic broth could be inoculated. After inoculation, the media should be placed in an anaerobic atmosphere as soon as possible. The best method for this incubation is the use of an anaerobic chamber. Other methods are being also used such as boxes, plastic envelopes shorter jars and automated gas flushing devices. The only caution using jars and boxes is that they should not be opened until after 48 h of incubation to prevent premature death of anaerobes. The period of incubation in the majority of cases should be of 5–7 days.

Identification of anaerobic bacteria

Initially, a presumptive identification could be performed with antibiotic disks such as vancomycin (5 μg), colistin (10 μg), penicillin (2 U) and kanamycin (1000 μg). Table 5 shows the presumptive identification of the main anaerobic microorganisms with the use of these antibiotic disks. There have been numerous ways in which bacteria could be identified: phenotypic (physiological/biochemical characteristics), chemical analysis (MALDI-TOF, nuclear magnetic resonance (NMR) spectroscopy), and genetic and molecular analysis (including nucleic acid sequencing). Until recently, biochemical methods have been used for genus and/or species identification. However, these methods are time consuming (1–5 days of anaerobic incubation), too cumbersome and costly for many laboratories (Holdeman et al., 1977). Now, there are simple and rapid micromethod identification systems which would permit laboratories with limited facilities to identify clinically important anaerobic bacteria (e.g. API ANA BioMérieux, ANI Card, BioMérieux, MINITEK Becton-Dickinson). Application of gas liquid chromatography (GLC) for the detection of anaerobes has been the confirmation method; however, the introduction of mass spectrometry (MALDI-TOF MS) in the routine of laboratories has supposed a great revolution in the diagnosis. This technique is a useful a simple method for rapid identification of microorganisms for the routine identification of clinical isolates (Nagy et al., 2009, 2012). Recent advances in detection of anaerobic microorganisms from clinical samples include 16S rRNA gene-based methods, DNA hybridization, multiplex polymerase chain reaction, and oligonucleotide array technologies (Coltella et al., 2013). 16S rRNA aids in taxonomic placement of sequences comparing the sequences to others (Song et al., 2003).

Laboratory diagnosis of *Clostridium difficile* infection

Isolation of the bacterium from liquid or semisolid stool specimens is necessary for epidemiological investigation including typing. Diagnosis of *C. difficile* infection has been based on the detection of the microorganism by culture in CCFA (cefoxitin-cycloserine fructose agar), CCEY (cefoxitin-cycloserine egg yolk agar) or CCEYL (cefoxitin-cycloserine egg yolk agar with lysozyme) and further demonstration of the toxins. Pretreatment of the stools with a similar volume of alcohol increases the isolation rate of the bacterium. After this, direct detection of the toxins from the isolated strain by the cytotoxic assay using different cell lines is necessary. However, at this moment, the most widely method used for the diagnosis is the detection of *C. difficile* specific antigens such as glutamine dehydrogenase (GDH), and toxin A and B, by immunocromatography or ELISA techniques. A diagnostic algorithm of *C. difficile* infection is showed in Fig. 2. More recently, real-time PCR methods have been developed to detect the

Table 5 Presumptive identification with antibiotic disks of main anaerobes.

Microorganism or group	*Vancomycin (5 μg)*	*Kanamycin (1000 μg)*	*Colistin (10 μg)*	*Penicillin (2 U)*
Gram-positive	S	V	R	V
Gram-negative	R	R[a]	S[b]	
B. fragilis group	R	R	R	R
Other *Bacteroides* species	R	R	V	S
Porphyromonas	S	R	R	
Fusobacterium	R	S	S	
Clostridium perfringens	S	S	R	
Prevotella	R	V	V	
Parabacteroides	R	R	R	

S = sensitive; R = resistant; V = variable.

[a]Some strains are sensitive.

[b]Unusual Gram-negative anaerobes may be resistant.

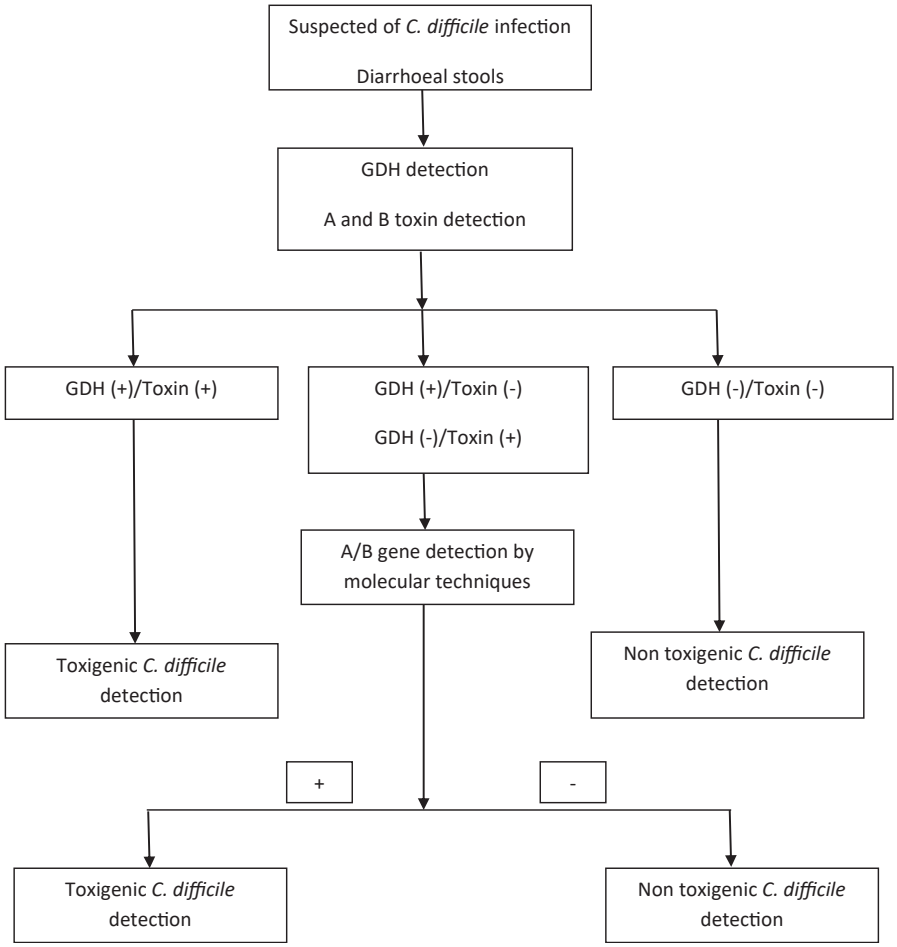

Fig. 2 Diagnostic algorithm of *C. difficile* infection.

toxin B gene (*tcdB*) directly from the stool or from the strain. Molecular methods for typing (PCR-ribotyping REA, PFGE, MLST) could be used for study of outbreaks.

Treatment of anaerobic infections: Antibiotic selection

Because anaerobic infections may cause abscess formation and tissue damage, therapy for these infections usually requires the administration of effective drugs, surgical debridement and/or abscess drainage. Moreover, as many of these infections are of mixed etiology (aerobes and anaerobes), the drugs used should be active against both aerobic and anaerobic microorganisms. Most anaerobic infections are treated empirically on the basis of some features such as the result of the Gram stain, the type of infection, the microorganisms often present in these infections, and the antimicrobial resistance pattern. Antimicrobial susceptibility testing of anaerobic bacteria is performed by a minority of laboratories (Goldstein and Citron, 2011; Smith et al., 2010) due to some circumstances like that cultures usually yield a polymicrobial flora or may be falsely negative. Current recommendations emphasize the fact that antimicrobial susceptibility testing of anaerobic isolates is only needed for severe infections, to test new antimicrobial agents or to monitor local and regional resistance patterns, although a rise in the resistance of anaerobes to some drugs may indicate a greater need for this testing (Brook et al., 2013; Schuetz, 2014).

Currently, the main drugs used against the majority of anaerobic infections include metronidazole, carbapenems, β-lactam/βlactamase inhibitor and clindamycin. Table 6 summarizes the spectrum of activity of various antibiotics that can be used against anaerobic pathogens.

Depending on the clinical entity, a specific antimicrobial treatment should be applied due to the predominant microorganisms that may be encountered. For intraabdominal infections, antimicrobial coverage of *Bacteroides* spp. and Gram-negative aerobic microbiota is mandatory. For patients with mild-to-moderate community-acquired infections single agents such as carbapenems and β-lactam/βlactamase inhibitor combinations are suitable. Other valid options could be tigecycline, moxifloxacin and

Table 6 Activity spectrum of main drugs used against anaerobic microorganims.

Antimicrobial agent	*Spectrum of activity/characteristics*
Penicillin, ampicillin and amoxicillin	Active against anaerobes that do not produce β-lactamase. Almost all GPACs are susceptible to them, but up to 80% of *B. fragilis* strains are resistant to penicillins. Many strains of *Prevotella* spp. and some Clostridia are also resistant.
Cephalosporins	Overall, this group has less activity in vitro than penicillin versus most anaerobes. Many strains of *B. fragilis* group, *Prevotella*, *Porphyromonas* and *Fusobacterium* species produce cephalosporinases. 85% of isolates are susceptible to cefoxitin. Cefoxitin has poor activity against Clostridia. Cefotetan is less effective than cefoxitin.
β-lactam/β-lactamase inhibitor	Good option against β-lactamase producing anaerobes. Ceftazidime-avibactam has limited action against anaerobic microorganisms.
Metronidazole	This drug is active against Gram-negative anaerobes, including the *B. fragilis* group. Inactive versus *Cutibacterium*, *Actinomyces* and microaerophilic streptococci. Penetrates well in abscesses.
Clindamycin	This antibiotic is active against many anaerobes. Rates of resistance have increased in last years. Some Clostridia other than *C. perfringens* are resistant.
Carbapenems	All components of this group of antibiotics are equally active against anaerobes. They are resistant to most *Bacteroides* β-lactamases.
Fluoroquinolones	The most active in this class for anaerobic infections is moxifloxacin. Increased resistance in *B. fragilis* group, so no recommended for intra-abdominal sepsis
Vancomycin	Active against Gram-positive anaerobes; inactive against Gram-negative anaerobes
Macrolides	They have moderate to good in vitro activity against anaerobic microorganisms; inactive against many *Fusobacterium* spp. and some *B. fragilis* spp.
Tigecycline	Active against some anaerobes, including strains of *B. fragilis* that are resistant to β-lactams, clindamycin and fluoroquinolones

GPACs: Gram-positive anaerobic cocci.

cefoxitin. A two-drug therapy with an antibiotic active against coliforms and the other against anaerobes can be an alternative. Clindamycin is now no longer recommended for intraabdominal infections because increasing rates of resistance in *B. fragilis* group.

For oral infections, drugs active against both anaerobic microbiota of the mouth and the Gram-positive aerobic microbiota should be applied. B-lactams antibiotics (e.g. penicillins, cephalosporins) alone are not suitable because the β-lactamase production of anaerobic microorganisms. Antimicrobial treatment for these infections may include β-lactam/βlactamase inhibitor combinations, clindamycin or penicillin along with metronidazole.

Antimicrobial susceptibility testing

Susceptibility testing should be performed for clinically relevant anaerobic microorganisms involved in human infections. The disk diffusion method is not recommended for anaerobic pathogens, so the gradient diffusion method using Etests is the choice method (CLSI, 2012). The anaerobic strains should be sub-cultures in Brucella agar supplemented with 5% laked sheep blood, hemin, and 10 μg/mL vitamin K1. Plates should be incubated in anaerobic atmosphere at 35–37 °C for 48 h. However, agar dilution is the gold standard in the case of MIC determination for anaerobes, although this method is not suitable for routine laboratories.

Antimicrobial resistance

Antibiotic resistance among anaerobic microorganisms has increased significantly over the past decades (Hecht, 2004; Karlowsky et al., 2012). However, resistance rates vary widely among different geographic regions. Regarding to *B. fragilis* group, resistance to penicillin may be observed in around 80–90% of isolates, while a higher proportion of the isolates (20%) are also resistant to amoxicillin-clavulanate. The overall resistance rate to carbapenems is very low (<1%), although some studies have reported higher rates (Snydman et al., 2011). The most significant changes last years in *Bacteroides* spp. have been the increase of resistance rate to clindamycin, ranging 30–50%. The most active drug against *Bacteroides* spp. is metronidazole and resistance to this antibiotic remains rare, although reports are emerging worldwide (Boyanova et al., 2015; Cobo et al., 2019).

Regarding to *Prevotella*, the resistance rate to penicillin is also increasing (Cobo et al., 2019), and their resistance rate to clindamycin is ranging 11 to 40%. Overall, *Prevotella* isolates found no resistance to metronidazole, although in some studies this resistance has been detected (Cobo et al., 2017a; Shilnikova and Dmitrieva, 2015).

On the other hand, very few isolates of *Fusobacterium* shows resistance to antibiotics, except for penicillin due to β-lactamase production.

Among *Clostridium*, *C. difficile* shows high resistance rate to imipenem (90%) and clindamycin (40%), but no resistance to metronidazole or vancomycin. Among other *Clostridium* species, resistance may be observed for all antimicrobials, except for imipenem. Non-spore-forming Gram-positive bacilli are intrinsically resistant to metronidazole, but they are highly susceptible to penicillin, β-lactams/βlactamase inhibitor, and carbapenems. The rate of resistance to clindamycin is highest for *Actinomyces* and lowest for *Cutibacterium*.

Finally, regarding to GPACs no resistance to carbapenems and β-lactams/βlactamase inhibitor may be observed in any genera. On the other hand, resistance to moxifloxacin is high in all genera, ranging between 25% and 46%. In this group of microorganisms,

the resistance rate to clindamycin is much higher for *Finegoldia* (around 50%) and *Peptoniphilus* (around 40%). Some GPAC strains have shown resistance to metronidazole (Cobo et al., 2017b).

In conclusion, routine antimicrobial susceptibility testing for anaerobes contributes information on the global situation and permits empirical therapies to be selected in accordance with local data on resistant strains.

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