Anaerobic bacteria infection in cystic fibrosis airway disease

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--- SUMMARY ---

Depletion of the periciliary liquid in "Cystic Fibrosis" airway disease results in reduced mucociliary transport, persistent mucus hypersecretion and consequently increased height of the luminal mucus layer, so hypoxic gradients in the mucus plugs are developed. Because of anaerobic lung zones, it is highly probable that anaerobic bacteria not detected by routine bacteriologic culture methods also reside within the mucus. Notwithstanding this evidence, microbiology laboratories working in the cystic fibrosis field do not generally use strict anaerobic bacteriologic cultures to determine the presence of anaerobic bacteria in the Cystic Fibrosis lung. The aim of this review is to focus on the published data regarding the finding of anaerobic bacteria in cystic fibrosis airway disease. Therefore, microbiology, diagnosis, antimicrobial susceptibility and possible impact on clinical management of anaerobic bacteria lung infection in cystic fibrosis are described.

KEY WORDS: Anaerobic bacteria, Chronic lung infection, Cystic fibrosis

INTRODUCTION

Cystic Fibrosis (CF) is the most common autosomal recessive disease in Caucasians (Ramsey, 1996). A hallmark of CF is chronic respiratory infection which may start very early in the lives of these patients. The mechanisms underlying the early acquisition of pulmonary infection in CF are very complex and currently remain unclear. At the basis of all hypotheses, the role of impaired muco-ciliary clearance related to low airway surface liquid volume is central, followed by the increased availability of cell surface receptors and impaired ingress of bacteria across epithelial cells (Pier *et al.*, 1996; Matsui *et al.*, 1998; Saiman and Siegel, 2004). Therefore, pulmonary infection has been recognized as having the greatest role in

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morbidity and mortality leading to premature death in 90% of patients (Armstrong *et al.*, 1997; Rosenfeld *et al.*, 2001; Dakin *et al.*, 2002; Rajan and Saiman, 2002).

Microbiology airway disease in cystic fibrosis

The epidemiology of pulmonary pathogens in the CF population has become more complex, and, although *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae* have been the most common pathogens in the lower airways, with improved survival, new emergent pathogens such as *Burkholderia cepacia* complex (Bcc), *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Aspergillus* spp, nontuberculous mycobacteria and respiratory viruses have been detected in the last years (Burns *et al.*, 1998; Goss *et al.*, 2002; Rajan and Saiman, 2002; Tan *et al.*, 2002; Bakare *et al.*, 2003; Olivier *et al.*, 2003; Saiman and Siegel, 2004; Lambiase *et al.*, 2006). Recently, other unusual bacteria such as *Acinetobacter* spp, *Bordetella* spp, *Moraxella* spp, *Comamonas* spp, *Rhizobium* spp, *Herbaspirillum* spp. and *Inquilinus limosus* have been described

(Coenye *et al.*, 2002), and Italian studies have reported Gram-negative non fermentative bacteria such as *Chryseobacterium meningosepticum, Chryseobacterium indologenes, Sphingobacterium spiritovorum* and *Sphingobacterium multivorum* in sputum samples of CF patients (Lambiase *et al.*, 2007; Lambiase *et al.*, 2009). Moreover, mycetes like *Scedosporium apiospermum* (Cimon *et al.*, 2000; Defontaine *et al.*, 2002) and *Penicillium* and *Exophiala* (Cimon *et al.*, 1999; Pihet *et al.*, 2009) have also been recognized.

The source of most pathogens in CF airway disease remains unknown, and potential reservoirs include the natural environment, the environment in health care settings, and, therefore, contaminated equipment and also other CF patients. Even with these findings, the impact on lung function is well recognized only for some pathogens such as *Pseudomonas aeruginosa*, Bcc, *Staphylococcus aureus*, whereas the relationship between other bacteria and clinical outcome, and the transmission of some pathogens have yet to be debated.

Defective ASL in cystic fibrosis airway disease

The negative impact of aberrations in transepithelial ion flow on the ionic composition and on the volume of airway surface liquid (ASL) in CF due to dysfunctional or absent CFTR has been well investigated over the last thirty years (Anderson and Welsh, 1992; Denning *et al.*, 1992; Welsh and Smith, 1993; Boucher, 2002). The ASL is a double layer on the epithelial surface: an upper mucus layer and a lower periciliary liquid layer (PCL) with a height of the extended cilium. The lower layer is regulated to maintain a low viscosity solution for the ciliary beat, while the upper layer is formed by high molecular weight mucins whose functions are altered by water content, ion concentration and pH. Therefore, in normal airway epithelia, the presence of normal PCL promotes efficient mucociliary clearance: consequently, the normal rate of cell oxygen consumption results in the absence of the gradient of partial oxygen pressure within the ASL (Knowles and Boucher, 2002).

In the CF airway, the aberration in ion flow achieves modifications in PCL volume and consequently a reduction of mucociliary transport and increased height of mucus plugs. The for-
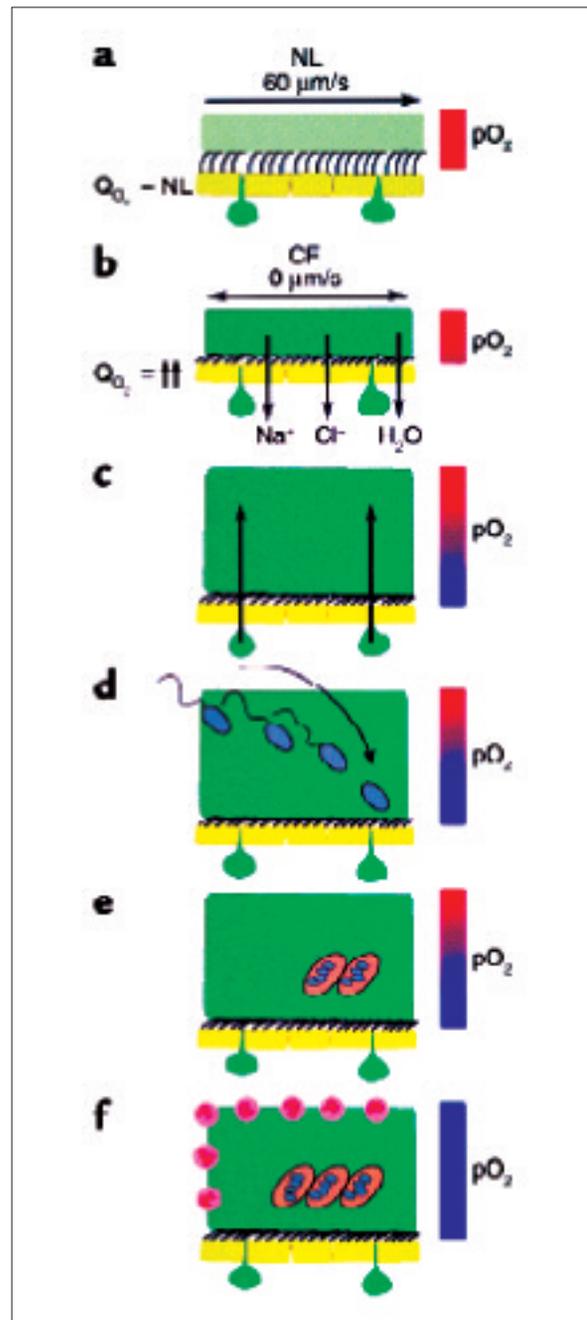

FIGURE 1 - *Pathogenic events hypothesized to establish chronic Pseudomonas aeruginosa infection in CF lung. (a) Normal volume and size of ASL and PCL promote an efficient mucociliary clearance. (b) In CF airway disease, there is a depletion of PCL and persistent mucus hypersecretion (c). Thus, hypoxic gradients in the mucus layer are generated and Pseudomonas aeruginosa can actively or passively penetrate into these anaerobic zones (d-e). The recruitment of neutrophils (f) induces a more hypoxic mucus layer, resulting in chronic airway infection (from Worlitzsch et al, 2002).*

mation of pO_2 gradient within ASL gives indications on the presence of local hypoxic zones within the mucus plaques (Figure 1, from Worlitzsch *et al.*, 2002). Oxygen tension plays an important role in the establishment of infection both by mutant strains and by anaerobic bacteria. In the airway, conditions such as hypoxia within mucus plugs may attract motile microrganisms capable of anaerobic survival and encourage biofilm formation. When the micro-colonies mature into the thickening biofilm, an oxygen gradient is generated, and oxygen levels are highest at the top of the biofilm and low/absent at the substratum. As chronic disease progresses, oxygen tension becomes dramatically reduced, and the CF airway mucus can be micro-aerobic or even anaerobic. In addition, several studies have demonstrated that the presence of hypoxic zones induces the expression of specific genes, such as the *alg* gene for alginate production in *Pseudomonas aeruginosa* and the consequent biofilm formation by this microrganism (Hentzer *et al.*, 2001). Recent evidence suggest that in the CF lung, bacteria such as *Pseudomonas aeruginosa* can grow when anaerobic conditions are established (Yoon *et al.*, 2002; Worlitzsch *et al.*, 2002), and the same anaerobic growth significantly enhances *Pseudomonas aeruginosa* biofilm formation and antibiotic resistance (Lyczak *et al.*, 2002).

Goals of the present review

Anaerobic bacteria are involved in a wide variety of human diseases, playing an important role in gastrointestinal infections, female genital tract infections and chronic upper and lower respiratory tract infections. Although anaerobic bacteria can often be isolated from clinical specimens, it is difficult to isolate them in pure culture, thus underestimating their importance in human disease.

Because of considerable heterogeneity in biochemical properties, several attempts have been made to devise a satisfactory classification of anaerobic bacteria. In recent years, many analytical techniques have been used to investigate the heterogeneity of anaerobic bacteria, both Gram-positive and Gram-negative. These techniques included DNA G+C content, DNA-DNA homology studies, comparison of 16S rRNA sequences, analysis of cell-wall fatty acids and peptidoglycan structure, whole-cell composition by pyrolysis mass spectrometry, analysis of 16S rDNA sequences, and analysis of 16S-23S intergenic ribosomal RNA polymorphisms.

Given the evidence of anaerobic conditions in the CF lung, and, above all, the well known question that in microbiological cultures of airway secretions of CF patients, bacteria can be easily missed if not specifically looked for, some studies have investigated the presence of anaerobic microrganisms in this anatomic district.

Therefore, the present review focuses on the published data regarding the finding of anaerobic bacteria in CF airway disease, including microbiology, diagnosis, antimicrobial susceptibility of these bacteria and possible impact on clinical management of these infections.

Microbiological diagnosis of anaerobic bacteria

Samples of choice

The bacteriological study of anaerobes in infectious processes is generally overlooked for several reasons. Among these, it is evident that anaerobic conditions are necessary during specimen transport. Several studies indicate the expectorated sputum, both natural and induced, as a respiratory sample for anaerobic bacteria research. This sample is more used because it is considered by many workers in this field an accurate indicator of lower airway microbiology, besides the preferred airway indicator for management of CF lung disease. However, the problems linked to this sampling are well known both for aerobic bacteria and anaerobic bacteria research. There are difficulties in some patients who do not expectorate: besides, the possibility of bacterial contamination of the upper respiratory tract is very likely.

In a recent study in 2009, Worlitzsch *et al.* (2009), studying a CF population during pulmonary exacerbation, indicated the expectorated sputum as the sample of choice for the search of anaerobic bacteria. Worlitzsch *et al.* analyzed 114 samples obtained from 45 CF patients, and 41 of these were infected by one or more obligate anaerobic species. To evaluate the possibility of oral contamination, they also took throat swabs of the pharynx, and obligate anaerobes in low numbers were found ($7.5x10^3 \pm 1.1x10^4$ CFU/ml, in contrast with mean values of $5.5x10^7$ CFU/ml for facultative anaerobes and $2.2x10^7$ CFU/ml for obligate anaerobes recovered in expectorated sputum) and only in three of seven patients.

In the study by Tunney *et al.* (2008), a total of 66 sputum samples were collected from 50 CF patients, and anaerobic bacteria were found in 42 of the 66 samples and in 33 of the 50 patients (10^4-10^7 CFU/ml). They also evaluated bronchoalveolar lavage fluid (BALF) to look for anaerobic bacteria, and a total of 10 BALF from 10 pediatric patients underwent strict anaerobic bacteriologic culture techniques. A total of 8/10 BALF were positive for aerobic and anaerobic bacteria but at lower numbers than in the sputum from adult CF patients. Based on these results, the authors suggested that it is likely due to the dilution of lung secretions (from 9 to 78 fold).

In the study by Harris *et al.* (2007), the authors used bronchoalveolar lavage and identified 65 different obligate anaerobic bacterial species from 28 CF children. Previously, in the study by Rogers *et al.* (2004), the bacterial community present in the oral cavity and in the lung of adult CF patients was investigated, and the authors identified various anaerobes in CF sputum, indicating that sputum samples were not subject to profound oral cavity bacterial contamination.

Other authors indicate the use of sputum samples to detect anaerobic bacteria in the CF lung, such as Jewes and Spencer (1990), who cultured 109 sputum samples obtaining anaerobic growth by 26 of these.

Although the use of expectorated sputum samples is indicated to search for anaerobic bacteria, other authors in the past investigated the use of more invasive techniques, such as thoracotomy samples (Thomassen *et al.*, 1984) and transtracheal aspirates (Brook and Fink, 1983), comparing these samples with expectorated sputum. In particular, thoracotomy samples correlated well with both aerobic and anaerobic bacteriologic results of expectorated sputum, while this was not the case for transtracheal aspirate, where only anaerobic bacteria were found.

Facultative and obligate anaerobic bacterial cultures
Sputum samples must be liquefied, mixed or sonicated (Jewes and Spencer, 1990) with equal volumes of 1% dithiothreitol before incubation at 37°C for 30 min (Tunney *et al.*, 2008). Sputum samples must be treated for semi-quantitative culture. For facultative anaerobic bacteria research, the sample is solubilised in phosphate-buffered saline (1:1) and then incubated in ten-fold dilutions on Columbia agar supplemented with sheep blood (10%) and incubated aerobically for 24 h at 37°C. For obligate anaerobic bacteria, the sample is incubated in ten-fold dilutions on brain-heart infusion agar and Schaedler agar, supplemented with sheep blood (5%) at 37°C for up to seven days in anaerobic conditions (80% nitrogen, 10% hydrogen, 10% carbon dioxide) (Worlitzsch *et al.*, 2009). Other authors used the same serial dilution but in quarter-strength Ringers lactate, supplemented with L-cysteine 0.05%. After this, one-hundred-microliter aliquots were spread onto anaerobic blood agar, kanamycin-vancomycin laked blood agar, phenylethylalcohol agar and nutrient agar (Tunney *et al.*, 2008).

To recognize the presence of anaerobic bacteria, the sensitivity to metronidazole as a simple screening method for separating Gram-positive anaerobic cocci from facultative anaerobic microrganisms can be used (Jewes and Spencer, 1990). This method is described by other authors (Edson *et al.*, 1982; Wexler and Finegold, 1988; Sheikh *et al.*, 1993): briefly, in the inoculum, a 5-µg metronidazole disk is placed, and bacterial presence is indicated by a zone of inhibition around the disk.

For BAL samples and other surgical samples described previously, they must be processed by the above indicated methods but, obviously, without liquefaction.

To determine the significant value of culture growth, the semi-quantitative assay is needed, and authors indicate the diagnostic cut-off at $>=10^4$ CFU/ml sputum (Jewes and Spencer, 1990; Tunney *et al.*, 2008; Worlitzsch *et al.*, 2009).

Identification strategy
For the identification strategy, both traditional phenotypic methods and molecular methods have been used. An important step for the identification of anaerobic bacteria is their differentiation from microaerophilic and capnophilic microrganisms. For this purpose, Jewes and Spencer identified Gram-negative anaerobic bacilli by tests of dye and bile tolerance, pigment production, sugar fermentation, indole production, gelatin digestion and aesculin hydrolis, based on the scheme indicated by Duerden *et al.* (Duerden *et al.*, 1980; Jewes and Spencer, 1990). Traditional

phenotypic methods are also used by Worlitzsch *et al.* and by Tunney *et al.* (Tunney *et al.*, 2008; Worlitzsch *et al.*, 2009).

Although semi-quantitative cultures should be carried out, this strategy is also extremely time-consuming, with the added danger of achieving poor growth if inappropriate anaerobic culturing conditions are applied. Molecular genetic methods may help in the identification of anaerobic bacteria in the CF lung. Therefore, in a study in 2004, Rogers *et al.* (Rogers *et al.*, 2004) disclosed various fastidious anaerobes in CF sputum by detection of specific 16S rRNA. In a subsequent study, Harris *et al.* (Harris *et al.*, 2007) identified the16S rRNA of 65 different obligate anaerobic bacterial species in bronchoalveolar lavage (BAL) from 28 CF children. More recently, Bittar *et al.* compared standard microbiological culture and phenotypic identification of bacteria in CF sputum to the use of 16S rDNA amplification, cloning and sequencing, showing a high number of anaerobic bacteria found by molecular methods, underestimated by traditional methods (Bittar *et al.*, 2008).

Antimicrobial susceptibility test
Several authors indicate the use of E-test strips to determine the susceptibility of anaerobic isolates obtained from CF sputum. Generally, the antibiotics tested are ampicillin, clindamycin, meropenem, metronidazole, piperacillin-tazobactam, and ceftazidime (Tunney *et al.*, 2008; Worlitzsch *et al.*, 2009). Minimum inhibitory concentrations (MICs) are read after incubation at 37°C for 48 h in anaerobic conditions. Breakpoints for the antibiotics are according to the Clinical and Laboratory Standards Institute Breakpoints for antimicrobial susceptibility of anaerobic bacteria, document M11-A7 (CLSI, 2007). The reference strain generally used is *Bacteroides fragilis* (Tunney *et al.*, 2008).

Anaerobic bacteria species recovered in the CF lung
Based on the above-mentioned identification strategies, several authors have detected anaerobic bacteria species in CF expectorated sputum or in other respiratory samples.
Brook indicated the recovery of strict anaerobic bacteria and aerotolerant bacteria, such as *Veillonella parvula*, *Bacteroides melaninogenicus*

and *Lactobacillus* spp, in four of six transtracheal aspirates obtained from pediatric CF patients (Brook and Fink, 1983). After one year, Thomassen *et al.* (1984) recovered anaerobic bacteria from thoracotomy samples. Jewes and Spencer isolated anaerobes from 26 of 109 sputum samples at 10^5 CFU/ml of sample (Jewes and Spencer, 1990). In this study, the anaerobic microrganism most isolated was *Bacteroides,* with the following species: *Bacteroides disiens*, *Bacteroides intermedius*, *Bacteroides ureolyticus*, *Bacteroides oralis*, and *Bacteroides asaccharolyticus*, besides, also Gram-positive anaerobic cocci and Veillonellae were recovered.

Tunney *et al.* revealed 14 different genera from 42 of 66 total sputum samples obtained from 50 CF patients (Tunney *et al.*, 2008). Here, the genera *Prevotella*, *Veillonella*, *Propionibacterium* and *Actinomyces* were most frequently isolated at 10^4 up to 9×10^7 CFU/g of sputum. Other genera isolated were both strict anaerobes, microaerophilic and oxygen tolerant bacteria. Anaerobes were cultured by 69% of samples, where *Pseudomonas aeruginosa* were also cultured, but they were also found in samples where *Pseudomonas aeruginosa* where not found. In this work, the authors also indicated the isolation of anaerobic bacteria in BAL obtained from 10 pediatric CF patients. BAL positive for bacteria that grew anaerobically were 5/8, and these belonged to the genera *Prevotella*, *Veillonella*, *Propionibacterium* and *Atopobium*.

More recently, Worlitzsh *et al.* (2009) in their study analyzing 114 sputum specimens obtained from 45 CF patients, indicated bacterial growth in 93 samples and obligate anaerobe growth in 22 CF patients. Among 168 isolates, 16 belonged to anaerobic genera. The CFU/g of sputum were between 2.2×10^7 and 6.9×10^7. Interestingly, up to four different species were found per sputum sample. The most represented anaerobic genera were *Staphylococcus* (*Staphylococcus saccharolyticus*), *Peptostreptococcus*, *Actinomyces*, *Veillonella*, and *Clostridium*, which were detected in 37, 34, 19, 15 and 12 different sputum specimens, respectively.

In the same year, Bittar *et al.* made an interesting study evaluating the proportion of bacteria present in respiratory samples of CF patients that was not detected by culture-based method (Bittar *et al.*, 2008). Thus, comparing the phenotypic

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TABLE 1 - *Summary of anaerobic bacteria (genera and species) recovered in the CF lung from several biological samples.*

Sample	Gram-positive anaerobic bacteria	Gram-negative anaerobic bacteria	Reference
Transtracheal aspirate	*Lactobacillus spp*	*Veillonella parvula* *Bacteroides fragilis* *Bacteroides melaninogenicus*	Brook and Fink, 1983
Sputum	*Gram-positive cocci*	*Veillonella spp* *Bacteroides disiens* *Bacteroides intermedius* *Bacteroides ureolyticus* *Bacteroides oralis* *Bacteroides asaccharolyticus* *Unidentified pigmented Bacteroides spp*	Jewes and Spencer, 1990
Sputum	*Actinomyces spp* *Propionibacterium spp* *Peptostreptococcus spp* *Bulleidia spp* *Bifidobacterium spp* *Gemella spp* *Lactobacillus spp* *Clostridium spp* *Staphylococcus saccharolyticus* *Anaerobic streptococci*	*Prevotella spp* *Veillonella spp* *Fusobacterium spp*	Tunney, 2008
Bal	*Propionibacterium spp* *Atopobium spp*	*Prevotella spp* *Veillonella spp*	Tunney, 2008
Sputum	*Peptostreptococcus spp* *Gemella morbillorum*	*Dialister pneumosintes* *(Bacteroides pneumosintes)* *Lachnospiraceae genomosp.* *Phorphyromonas spp* *Prevotella denticola* *Prevotella melaninogenica* *Prevotella oris* *Prevotella salivae* *Selenomonas infelix* *Selenomonas noxia* *Selenomonas spp* *Tannerella forsythensis* *Veillonella atypica* *Veillonella spp*	Bittar, 2008
Sputum	*Staphylococcus saccharolyticus* *Peptostreptococcus anaerobius* *Peptostreptococcus micros* *Peptostreptococcus prevotii* *Peptostreptococcus tetradius* *Actinomyces israelii* *Actinomyces meyeri* *Actinomyces naeslundii* *Actinomyces odontolyticus* *Actinomyces turicensis* *Clostridium bifermentans* *Clostridium butyricum* *Clostridium clostridioforme* *Clostridium difficile* *Clostridium hastiforme* *Clostridium innocuum* *Clostridium perfringens* *Clostridium sporogenes* *Streptococcus constellatus* *Streptococcus intermedius* *Gemella morbillorum* *Eubacterium aerofaciens* *Eubacterium limosum* *Lactobacillus acidophilus* *Lactobacillus jensenii* *Propionibacterium acnes* *Propionibacterium granulosum* *Propionibacterium propionicum*	*Veillonella spp* *Bacteroides tectum* *Bacteroides stercoris* *Mobiluncus curtsii* *Mobiluncus mulieris* *Capnocytophaga spp* *Prevotella corporis* *Prevotella melaninogenica* *Fusobacterium necrophorum* *Wolinella spp*	Worlitzsch 2009

identification of bacteria to molecular methods using 16S rRNA amplification, cloning and sequencing, the authors demonstrated that the complex bacterial community in the CF lung, especially anaerobic bacteria, is underestimated due to inappropriate search methods. On 25 sputa, 33 isolates belonging to 13 species were recovered using classic microbiological cultures, while 53 different bacterial species, including 16 species of anaerobes were detected using a molecular assay. Among anaerobic species, *Porphyromonas spp*, *Prevotella spp*, *Prevotella melaninogenica* and *Veillonella* spp were the most represented. Table 1 summarizes the anaerobic bacteria recovered in several samples.

Antimicrobial susceptibility

Anaerobic bacteria are generally susceptible to a wide range of antibiotics. Antibiotics such as vancomycin, rifampicin, chloramphenicol, penicillins, cefotaxime, cefoxitin, carbapenems and β-lactams with β-lactamase inhibitors are active against these bacteria (Garcia-Rodriguez *et al.*, 1995). Moreover, studies have indicated that strictly anaerobic cocci are highly susceptible to

metronidazole, whereas other anaerobic bacteria such as *Propionibacterium* and *Peptostreptococcus* are resistant to metronidazole (Panichi *et al.*, 1990; Wexler *et al.*, 1993). Tetracycline, erythromycin and clindamycin also appear to have intermediate values of activity, and selection of clindamycin-resistant strains in course of therapy is reported (Ohm-Smith *et al.*, 1986). Resistance to glycopeptides has not been reported, and teicoplanin is more active with respect to vancomycin.

In the study by Tunney *et al.*, (2008) meropenem is reported as the only antibiotic for which the MIC_{90} fell within the susceptible range. Isolates of *Propionibacterium* are the most resistant to metronidazole (MIC_{50}>256 mg/L), isolates of *Veillonella* are the most resistant to piperacillin/tazobactam (MIC_{50}>256/4 mg/L) and some isolates of *Prevotella* are resistant to ampicillin, clindamycin and metronidazole. Worlitzsch *et al.* (2009) indicate a low rate of resistance to meropenem and a poor activity of ceftazidime in their study.

Results of some studies on antimicrobial susceptibility of anaerobic bacteria are showed in Table 2.

TABLE 2 - *Summary of antimicrobial susceptibility of anaerobic bacteria reported in two studies.*

Genera or species	No. of strains tested	No of resistant strains vs						Reference
		TZP	MTZ	CLI	MEM	CAZ	AMP	
Prevotella	14	1	7	5	0		5	Tunney, 2008
Veillonella	5	4	1	0	0		2	
Propionibacterium	5	0	4	0	0		1	
Actinomyces	8	0	3	1	0		3	
Peptostreptococcus spp	32	2	26	5	0	7		Worlitzsch, 2008
S. saccharolyticus	25	14	2	1	1	24		
Actinomyces spp	18	0	9	7	2	8		
Veillonella spp	14	8	4	1	0	11		
Streptococcus spp	10	0	10	4	0	2		
Clostridium spp	9	0	3	4	1	4		
Bacteroides spp	8	0	0	3	0	6		
Propionibacterium spp	3	0	3	0	0	0		
Lactobacillus spp	3	0	0	3	0	0		
Eubacterium spp	3	0	2	1	0	3		
Gemella morbillorum	3	0	2	2	0	1		
Prevotella spp	3	0	0	0	0	2		
Mobiluncus spp	3	0	2	0	0	0		
Capnocytophaga spp	2	0	2	1	1	1		
Wolinella spp	2	2	2	0	0	2		

TZP = piperacillin-tazobactam; MTZ = metronidazole; CLI = clindamycin; MEM = meropenem; CAZ = ceftazidime; AMP = ampicillin.

Impact of anaerobic bacteria infection in CF pulmonary disease

Concerning the epidemiology of anaerobic lung infection, Worlitzsch *et al.* (2009) indicate in their study, that the large variety of obligate anaerobic genera is present independent of the age of patient.

These anaerobic bacteria could come from the oral cavity, and, subsequently, install themselves in the lung because of favorable conditions for their growth. Worlitzsch *et al.* recovered a low number of obligate anaerobes in throat swabs of CF patients, indicating the oral cavity as the niche from which obligate anaerobes spread into the lower airway. On the other hand, Rogers, in a study in 2006, identified the oral cavity as the "stepping-stone" for lung colonization (Rogers *et al.*, 2006). In CF airway disease, the relationship between anaerobic conditions and *Pseudomonas aeruginosa* infection has been demonstrated. In spite this evidence, Tunney *et al.* demonstrated the presence of anaerobic bacteria in pediatric BAL even in absence of *Pseudomonas aeruginosa* infection (Tunney *et al.*, 2008; Pamukcu *et al.*, 1995; Palmer *et al.*, 2007). In a 2005 study, Hill *et al.* (2005) reported the efficacy of antibiotic combinations against *Pseudomonas aeruginosa* grown under anaerobic conditions, illustrating that antibiotic susceptibility is dependent on culture conditions. The presence of anaerobic bacteria has been demonstrated both during the quiescent phase of pulmonary disease (Jewes and Spencer, 1990) and during pulmonary exacerbations (Worlitzsch *et al.*, 2009): although the presence of bacteria in the quiescent phase of disease suggests a colonizing rather than a pathogenic role, the study by Worlitzsch *et al.* indicated that during exacerbations patients were infected by anaerobic bacteria resistant to the antibiotics used for pulmonary disease.

Tai and Ranganath, in a letter published in 2008, emphasized the importance of airway inflammation in CF lung disease (Tai and Ranganath, 2008). Thus, they indicate the importance of associating the presence of anaerobes in the CF lung with markers of pulmonary inflammation and also with the clinical management of general pulmonary disease.

Concluding remarks

The studies described in the present review are indicative of a part of clinical microbiology frequently underrepresented for several reasons, such as special culture conditions and extremely slow growth of colonies. Consequently, the use of molecular methods, such as 16S rRNA, PCR-RFLP, are strongly endorsed (Nagy *et al.*, 2006). Notwithstanding these studies, the clinical significance of anaerobes is unclear, but, because of their demonstrated presence in CF pulmonary samples, they may well contribute to the pathophysiology of CF airway disease.

In conclusion, taken together, these data indicate that infection in the CF airway is poly-microbial, therefore, further investigations on anaerobic bacteria infection in the CF lung can improve the management of CF patients, particularly helping to determine the best anti-microbial treatment.

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