Abstract

Anaerobic bacteria can cause an infection when they encounter a permissive environment within the host. These opportunistic pathogens are seldom recovered as single isolates but more frequently are involved in polymicrobial infections, together with other anaerobes or aerobes. Nowadays it’s known that some anaerobic bacteria are also able to grow as biofilm even if this feature and its role in the healthcare-associated infections (HAIs) are still poorly characterized. As consequence, the involvement of biofilm-forming anaerobic bacteria in infections related to healthcare procedures, including surgery and medical devices implantation, is underestimated.

The current knowledge on the role of biofilm-growing anaerobes in HAIs has been here reviewed, with particular reference to respiratory, intestinal, intra-abdominal, wound, and urogenital tract infections. Even if the data are still scarce, the ability to form biofilm of opportunistic anaerobic species and their possible role as causative agents of HAIs should alert even more clinicians and microbiologists on the need to search for anaerobes in clinical samples when their presence can be reasonably assumed.

6.1 Introduction

The increased recovery of anaerobes from clinical samples has led to a greater appreciation of their role in infections at virtually all body sites, including mouth, lung, gastrointestinal tract, urogenital tract, bloodstream, skin and soft tissue, and of their involvement in a variety of clinical presentations, such as abscess formation, foul-smelling pus, and tissue necrosis (Finegold 1995a).

In principle, anaerobic bacteria can cause an infection, becoming opportunistic pathogens, when they encounter a permissive environment within the host, subsequent to breakdown of the common barriers as a result of surgery, injury, blood vessel disease or shock. In fact, tissue destruction (necrosis) or poor blood supply can favour the growth of anaerobic bacteria because of the resulting low oxygen conditions (Finegold 1995b). Other predisposing factors include malignancy,
immunodeficiency, diabetes and presence of foreign bodies (Castillo et al. 1999).

These opportunistic pathogens are seldom recovered as single isolates, such as Finegoldia magna that is quite often isolated in pure culture (Wildeboer-Veloo et al. 2007), but more frequently are involved in polymicrobial infections, being isolated together with other anaerobes or aerobes (Nichols and Florman 2001; Brook 2002; Dryden 2010). Usually, many infections are initially caused by aerobic bacteria and then worsened by anaerobes that become predominant when the tissue microenvironment turn out to be anaerobic.

In a study by Mikamo and co-workers covering the years 1994–2003, it has been demonstrated that the most often isolated strains in polymicrobial infections are Gram-positive anaerobic cocci (25–30 %), followed by Prevotella spp., Bacteroides fragilis group, Clostridium spp., Veillonella spp., Fusobacterium spp. and Porphyromonas spp. (Mikamo et al. 2011). The major role of gram positive anaerobic cocci in mixed infections has been recently confirmed by other authors (Murphy and Frick 2013).

Other than the high number of endogenous anaerobic species commonly inhabiting our body and possibly causing infections, there are few anaerobes, first of all Clostridium difficile, able to cause endogenous or exogenous infections (Spigaglia et al. 2011; Wiegand et al. 2012; Knight and Sarawicz 2013).

The three major virulence factors supporting anaerobes in host adhesion and invasion are: (i) the production of toxins or enzymes such as superoxide dismutase, catalase, immunoglobulin proteases (Mastrantonio et al. 1996); (ii) the surface structures such as the lipopolysaccharide or the capsular polysaccharide, that are often expressed only in chronic infections (Brook et al. 1991), (iii) the ability to adhere to or invade epithelial surfaces (Hofstad 1989; Brook and Frazier 1993; Brazier 2006).

Besides the above mentioned virulence factors, nowadays it’s known that some anaerobic bacteria are also able to grow as mono- (Fig. 6.1) or dual-species biofilms (Donelli et al. 2012) even if this feature and its role in the healthcare-associated infections (HAIs) are still poorly characterized.

In fact, although it has been well demonstrated the close association between biofilm-forming anaerobic species and oral diseases, such as peri-implantitis, the involvement of biofilm-forming anaerobic bacteria in infections related to surgery, devices implantation or other healthcare procedures is underestimated.

The current knowledge on the role of biofilm-growing anaerobes in HAIs will be here reviewed, with particular reference to respiratory, intestinal, intra-abdominal, wound, and urogenital tract infections.

### 6.2 Lower Respiratory Tract Infections

According to the American Thoracic Society and Infectious Diseases Society of America (2005), the lower respiratory tract infections, including hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), are the most common HAIs in acute care hospitals, causing significant morbidity and mortality in hospitalized patients (Chroneou et al. 2007; Werarak et al. 2010).

In healthy people, thanks to the cleansing action of the ciliated epithelium, the lower respiratory tract (trachea, bronchi, and pulmonary tissues) is virtually free of microorganisms that are pushed upward and removed by coughing, sneezing, swallowing, etc.

When mechanical or chemical injuries to the ciliated epithelium affect the normal mucus removal, the patient may become susceptible to infection by pathogens, including *Streptococcus pneumoniae* and several nosocomial multidrug resistant bacteria, such as the aerobes *Acinetobacter baumannii, Haemophilus influenzae, Klebsiella pneumoniae* and the facultative anaerobes *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ferrara 2006; Spronk 2007).

However, most of the lower respiratory tract infections are polymicrobial and some of them, such as aspiration pneumonia or ventilator-associated pneumonia, include also strictly anaerobic bacterial flora. The predominant anaerobic bacteria were the pigmented *Prevotella* spp., other than *Actinomyces, Bacteroides, Peptostreptococcus, Veillonella, Propionibacterium, Fusobacterium*...
spp. and Porphyromonas spp. (Marik and Careau 1999; Robert et al. 1999; Brook 2004a; Bahrani-Mougeot et al. 2007; Bartlett 2012).

Particularly, VAP is the most common intensive care unit (ICU)-acquired infection in patients requiring prolonged mechanical ventilation and it occurs in 8–28% of patients (Agbaht et al. 2007; Choudhuri 2013). Furthermore, VAP, from the economic side, is associated with higher costs due to the longer hospital stay (Gould 2013).

The endotracheal tube (ETT) is considered one of the major risk factors for VAP, altering the patient’s ability to clear secretions by coughing, thus allowing their passage into the airways, and acting as a reservoir for potentially infecting microbes and as a bridge between the oropharyngeal environment and the sterile bronchoalveolar space (Koerner 1997).

One of the most important mechanisms implicated in the development of VAP is the biofilm formation on the ETT surfaces. In fact, shortly after intubation, the ETT may represent a source of pathogens by providing inner and outer luminal surfaces to which microbes can adhere and form biofilms, thus contributing to pathogenesis and persistence of colonization (Bauer et al. 2002; Zur et al. 2004; Pneumatikos et al. 2009; Zolfaghari and Wyncoll 2011; Vandecandelaere et al. 2012).

Once biofilm on ETT is formed, there are several mechanisms by which it can infect the lungs: microbial clusters may be dispersed and
passively moved towards the lungs, cell aggregates can be aerosolized and aspirated in the airways and individual cells in contact with liquids can be transferred deeply into the lungs (Luna et al. 2009).

Even if the bronchoalveolar lavage cultures are considered the ‘gold standard’ for the identification of respiratory pathogens causing VAP, this procedure is not able to identify all the potential pathogens constituting the ETT biofilm. In fact, two independent groups (Perkins et al. 2010; Cairns et al. 2011) recently explored the bacterial community adherent to the ETT surfaces through molecular techniques, both of them evidencing the presence of an anaerobic component of the oral cavity.

Perkins and co-workers examined eight ETTs recovered after intubation periods between 12 h and 23 days from patients admitted in a surgical and a medical intensive care unit. To identify and quantify the fastidious/non culturable organisms present within the multi-species biofilm of the investigated ETTs, 16S rRNA gene survey and quantitative polymerase chain reaction (qPCR) were performed. The results showed that, on a number of 1263 near full-length 16S rRNA gene sequences from the diverse bacterial communities, the second most frequent genus identified corresponded to the anaerobe Prevotella spp. (179/1263), with the highest relative concentrations for the ETT tubes with short intubation periods. This study firstly demonstrated the presence of anaerobic oral bacteria directly within the ETT biofilms (Perkins et al. 2010). Afterwards, Cairns and coworkers also demonstrated that oral obligate and facultative anaerobic bacteria form part of the ETT biofilm. In fact, by using species specific PCR, the obligate anaerobe Porphyromonas gingivalis and the facultative anaerobe Streptococcus mutans were detected within the polymicrobial biofilm grown on the ETTs of 9 out of 20 patients (Cairns et al. 2011).

The detection of oral anaerobic species has demonstrated that oral biofilm may play a considerable role in lower respiratory tract infections. Oral decontamination, i.e. by the use of chlorhexidine-based oral hygiene, in conjunction with VAP prevention bundle, is effective in reducing the incidence of VAP by 30 % and the duration of mechanical ventilation in patients in the surgical ICU (Genuit et al. 2001; Chlebicki and Safdar 2008).

Considering the ability of oral anaerobic species to form a polymicrobial biofilm (Marsh 2004; Kolenbrander et al. 2010; Roberts and Mullany 2010; Zijnge et al. 2012) and the increasing number of studies suggesting a potential role of anaerobes in respiratory diseases, particularly in cystic fibrosis (Costerton 2002; Worlitzsch et al. 2009; Ulrich et al. 2010; Su and Hassett 2012), the direct involvement of anaerobes in the pathogenesis and persistence of these biofilm-based HAIs has to be taken into consideration and further investigated.

### 6.3 Intestinal Infections

The gastrointestinal (GI) tract of the normal healthy humans harbours a complex indigenous flora, mostly anaerobes within the colon, that plays a crucial role in the maintenance of normal metabolic and immunologic homeostasis (Savage 1977).

These mucosal communities are characterized by a sessile mode of growth, rather than a non-adherent planktonic state, with different fermentation profiles and enzymatic activities significantly higher in sessile-growing bacteria (Probert and Gibson 2002; Zoetendal et al. 2002; Macfarlane and Macfarlane 2006).

Microscopic investigation of the colonic mucosa by using specific 16S rRNA fluorescence in situ hybridization (FISH) probes, has confirmed that mucosal bacteria, including enterococci, bacteroides and bifidobacteria, are distributed throughout the mucus layer and occur extensively in microcolonies. Live/dead staining of these structured communities showed that most of the bacteria were living, particularly those closest to the mucosal surface. These findings suggest that the bacteria are actively growing in the mucus layer, and that their presence is not a result of passive transfer of the cells from faecal material along the gut lumen (Macfarlane and Dillon 2007; Macfarlane et al. 2011).

Due to their proximity to the epithelial surface, mucosal bacteria growing in biofilm may be
important in modulating the host’s immune system and possibly contributing to some inflammatory bowel diseases (ulcerative colitis, Crohn’s disease), in which exists a dysbiosis in microbial community structure, with a reduction in putatively protective mucosal microorganisms such as bifidobacteria (Macfarlane et al. 2011).

Sproule-Willoughby and coworkers studied selected representatives from the human colonic microbiota by using mucosal bacterial communities from the human colon and allowing them to grow in a surface-adherent mode of growth. The resulting biofilms were complex, multi-species communities, stable in composition over an extended period. This model is useful for investigating the effects of exogenous microbial, environmental and pharmaceutical influences on bacterial community structure and function in the intestine (Sproule-Willoughby et al. 2010).

Different intestinal anaerobic isolates, belonging to the species Bacteroides, Clostridium, Fusobacterium, Finegoldia, Prevotella, and Veillonella have been demonstrated to be able to in vitro adhere, to develop as mono-species biofilms, and to interact with each other giving rise to dual-species biofilms (Donelli et al. 2012).

The healthcare-associated intestinal infections are mainly related to the increased use of broad-spectrum antibiotics that are able to promote abnormal gut colonization by resistant pathogens. In fact, the disruption of the anaerobic flora has been shown to be a key factor for the gut colonization by the anaerobic-facultative vancomycin-resistant Enterococcus sp (Donskey et al. 2000) and C. difficile (Lo Vecchio and Zacur 2012), both species being strongly associated with hospital outbreaks and invasive infections.

Many strains of Enterococcus faecium subpopulation belonging to the clonal complex 17 (CC-17) contain a putative pathogenicity island encoding a variant of enterococcal surface protein (Esp). Esp expression depends on growth conditions like temperature and anaerobiosis, this protein being found in half of these strains grown at 37 °C under anaerobic conditions. Furthermore, amounts of surface-exposed Esp was shown to correlate with initial adherence to polystyrene (R(2)=0.7146) and biofilm formation (R(2)=0.7535). These data indicate that E. faecium senses and responds to the change of environmental conditions, which might play a role in the early stages of infection when bacteria transit from oxygen-rich conditions at room temperature to anaerobic conditions at body temperature (Van Wamel et al. 2007).

Regarding the most insidious intestinal HAI, i.e. the Clostridium difficile infection (CDI), different research groups have started to study C. difficile biofilm. Donelli and coworkers first demonstrated the in vitro ability of a C. difficile clinical strain to grow as biofilm, alone or synergistically developing together with a F. magna strain (Donelli et al. 2012).

Afterwards, the hypervirulent strain R20291 was revealed to grow as biofilm and a possible link between sporulation and biofilm formation was suggested, by putting into evidence a reduction of biofilm formation in a spo0A mutant (Dawson et al. 2012).

A deeper analysis on the strain 630 and the hypervirulent strain R20291 conducted by Dapa and coworkers confirmed the ability of the C. difficile hypervirulent strain to form biofilm and, employing isogenic mutants, authors showed that the virulence-associated proteins, Cwp84, flagella, and a putative quorum-sensing regulator, LuxS, are all required for a maximal biofilm formation. It has been also demonstrated that bacteria in clostridial biofilms are more resistant to high concentrations of vancomycin, a drug commonly used for CDI treatment (Dapa et al. 2013; Dapa and Unnikrishnan 2013).

On the whole, the above mentioned data suggest that biofilm formation by C. difficile (Figs. 6.2 and 6.3) is a complex multifactorial process, modulated by several different factors, that could play a key role in gut colonization and bacterial survival, thus possibly affecting its pathogenesis and persistence, and contributing to recurrence of CDI.

### 6.4 Intra-Abdominal Infections

Healthcare-associated intra-abdominal infections affect a spectrum of adult patients receiving cares in acute hospitals or residing in chronic care settings. Cultures of the peritoneal cavity of patients affected by peritonitis due to a post-operative

complications, anastomotic leaks or device-related infections, such as continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis and infected ventriculoperitoneal shunt, allow to identify the polymicrobial nature of this infection (Brook and Frazier 2000; Marshall 2004; Mazuski and Solomkin 2009).

In fact, patients more often at risk for infection with multidrug resistant (MDR) bacteria, are typically infected with Escherichia coli, P. aeruginosa and Acinetobacter spp., extended spectrum beta-lactamase (ESBL)–producing Klebsiella spp., Enterobacter spp., Proteus spp., and Enterococci. In addition, the involvement of anaerobes in these infections has been also demonstrated (Sartelli et al. 2012).

In case of post-operative complications, after an initial stage of infection caused by aerobes and characterized by preliminary disruption of intra-abdominal hollow viscera and decrease in the oxidation-reduction potential of the oxygenated tissue, the anaerobic B. fragilis starts to predominate in one third to one half of these infections (Goldstein and Snydman 2004; Marshall 2004; Brook 2008).

On the contrary, in CAPD-associated peritonitis the typical spectrum of microorganisms include gram-positive (67 %) and gram-negative (28 %) aerobic bacteria, and a low percentage of anaerobic microorganisms (2.5 %) (Troidle and Finkelstein 2006; Chao et al. 2013). A polymicrobial biofilm as cause of this infection has been deeply analyzed (Verger et al. 1987; Ward et al. 1992; Gorman et al. 1994 Dasgupta and Larabie 2001; Dasgupta 2002; Hanlon et al. 2004; García-López et al. 2012; Nessim et al. 2012; Martins et al. 2013) and the presence of an anaerobic component has been revealed in some investigated peritoneal catheters (Troidle and Finkelstein 2006; Pihl et al. 2013).

Troidle and coworkers examined microbial biofilms grown on ten peritoneal catheters removed from eight patients because of peritonitis and from other two patients because no longer
needed. Among the microorganisms identified within the biofilm, B. fragilis represented the most frequently isolated anaerobe (Troidle and Finkelstein 2006).

Literature data have demonstrated the ability of B. fragilis to form in vitro biofilm (Weinacht et al. 2004) and to grow on and colonize mucin surfaces (Macfarlane et al. 2005) as well as its capability to enhance bacterial co-aggregation under bile salt exposure by overexpressing several bmeB efflux pumps and the outer membrane protein Omp (Pumbwe et al. 2007).

These findings support the hypothesis that biofilm-growing B. fragilis could contribute to cause intra-abdominal infections due to the insertion of peritoneal catheters.

By using standard microbiology methods and 16S rRNA gene sequencing, Pihl and coworkers set out to identify the range of aerobic and anaerobic bacterial species on CAPD catheters from patients with or without infections. Bacteria were found heterogeneously spread on catheters, both as single microorganism or mixed microbial communities. The most common colonizer was Staphylococcus epidermidis, followed by the anaerobic species Propionibacterium acnes, the latter being widely spread over the surface of colonized catheters (Pihl et al. 2013).

Another biofilm-based infection has been related to the failure of biliary decompression after endoscopic insertion of a plastic stent in patients suffering from obstructive jaundice. In fact, the device occlusion (Fig. 6.4) has been first described as consequence of the deposition of biliary sludge (McAllister et al. 1993; Weickert et al. 2001) while, later, it has been reported that microbial biofilm plays a pivotal role in the clogging process (Jansen et al. 1993; Hoffman et al. 1994; Sung 1995; Brant et al. 1996; Leung et al. 1998; Zhang et al. 2002; van Berkel et al. 2005; Donelli et al. 2007; Weickert et al. 2009).

Leung and coworkers demonstrated for the first time the presence of anaerobic bacteria, especially Clostridium perfringens, Clostridium bifermentans and B. fragilis, in the biliary stents’ biofilm, and their contribution in initiating stent blockage in patients who had received antibiotic prophylaxis against gram-negative bacterial infection (Leung et al. 2000).

Afterwards, several species of strictly anaerobic bacteria were observed in a couple of papers. Particularly, Scheithauer and coworkers evidenced Fusobacterium spp. and Veillonella spp. (Scheithauer et al. 2009) while Guaglianone and co-workers have reported the isolation of strictly anaerobes from 57 % of the investigated biliary stents, Bacteroides spp. and Clostridium spp. being the most represented anaerobic species, followed by Prevotella spp., Veillonella spp., Fusobacterium spp. and Peptostreptococcus spp. (Guaglianone et al. 2010).
6.5 Wound Infections

Infected wounds are damaged area of the body colonized by bacteria or other microorganisms that, depending on their pathogenicity and inoculum size, overwhelm the body’s immune defences, producing either a delay in wound healing or deterioration of the wound.

The onset of chronic wounds, that are lesions failed to proceed through an orderly and timely restore to health, is the main cause of the delay in the healing process (Edwards and Harding 2004; Thomson 2011).

The occurrence of chronic wounds has been related to the presence of highly persistent biofilm communities enabling microbial escape from host immune system and resistance to antibiotic treatment (James et al. 2008; Wolcott et al. 2008; Percival et al. 2012).

Infections that occur in the wound site at the end of an invasive surgical procedure are generally referred to as surgical site infections (SSIs). They represent about a fifth of all HAIs and are an important cause of morbidity and mortality, over one-third of postoperative deaths being related, at least in part, to SSIs (Mangram et al. 1999; Bansal et al. 2005; Kiernan 2012).

Viable biofilms have been associated with both monofilament and braided infected sutures, and associated reactive soft tissue (Kathju et al. 2009; Edmiston et al. 2013).

Microorganisms causing SSIs are usually derived from the patient (endogenous infection), but also exogenous infection may occurs when microorganisms from the instruments and external environment contaminate the operative site (Bowler et al. 2001). Even if S. aureus is the microorganism most commonly cultured from SSIs, wounds are very often infected by a whole range of microorganisms (National Collaborating Centre for Women’s and Children’s Health. Clinical Guideline, October 2008). In fact, it is considered that also other aerobic or facultative pathogens such as coagulase-negative staphylococci, P. aeruginosa, E. coli, Klebsiella spp., Enterobacter spp., Enterococcus spp. and beta-hemolytic Streptococci, as well as Candida spp., are the primary causes of delayed healing and infection in chronic wounds, especially the surgical ones (Mangram et al. 1999).

Furthermore, Wolcott and coworkers specified that over 60 % of the bacteria in the evaluated SSIs were anaerobic bacilli while the previous literature data indicates that aerobic cocci predominate in such wounds (Wolcott et al. 2009).

Also the healthcare-associated pressure ulcers, defined as localized injury to the skin and underlying tissue usually as a result of pressure occurring in immobilized patients, are often associated to infections caused by polymicrobial biofilms, with no single bacterial species exclusively colonizing the wounds (Ebright 2005; James et al. 2008; Smith et al. 2010).

Today, approximately 20 % of long-term care patients suffer from infected pressure ulcers (Zulkowski et al. 2005; Donelli and Vuotto 2014).

A multi-faceted approach constituted by 16S rRNA pyrosequencing, epifluorescence microscopy, FISH, and quorum sensing analysis, is today available to identify the entire spectrum of bacterial species and to fully characterize the microbial complex nature of chronic wounds (Han et al. 2011).

In fact, three separate 16S-based molecular amplifications followed by pyrosequencing, shotgun Sanger sequencing, and denaturing gradient gel electrophoresis have allowed to survey the whole biofilms-forming bacterial populations in pressure ulcers. Results showed that obligate anaerobes represented 62 % of the investigated microbial populations (Dowd et al. 2008).

Consistent results have been obtained by Smith and colleagues in 2010, many of the analyzed wounds being predominated by what are either facultative or obligate anaerobic bacteria with only 36 % of aerobes. The most frequently isolated strict anaerobe was F. magna (32/49 decubitus ulcer samples), followed by Anaerococcus vaginalis (23/49), Anaerococcus lactolyticus (20/49), Peptoniphilus indolicus (20/49), Peptoniphilus harei (18/49), Peptoniphilus ivorii (17/49), Peptoniphilus lacrimalis (13/49), Porphyromonas somerae (13/49), Prevotella buccalis (12/49). The anaerobic species B. fragilis, Porphyromonas spp., and Prevotella bivia were isolated in lower number (Smith et al. 2010).
The development of these aerobic-anaerobic populations is facilitated by the low oxygen tension (hypoxia or anoxia) and the reduced redox potential of the wound environment (Gerding 1995).

The high prevalence of anaerobic bacilli detected today suggest that the complexity of bacterial communities in wounds has historically been underestimated and that these bacterial species may be leading contributors to the aetiology of biofilm-related chronic wound infections.

### 6.6 Urogenital Infections

Urinary tract infections (UTIs) are the most common HAIs in the intensive care units (Shuman and Chenoweth 2010).

It has been recently estimated (Cek et al. 2014) that around 10 % of hospitalized urological patients are at risk to develop UTIs often caused by multiresistant uropathogens, such as enteric Gram-negative bacilli, enterococci, Candida species, and *P. aeruginosa*, *E. coli* being the most frequent isolate (544 of 1,371 isolates; 39.7 %).

Persistent or recurrent UTIs predominantly occur in patients with indwelling urinary catheters prone to be colonized by different bacteria, catheter-associated urinary tract infections (CAUTIs) accounting for approximately 40 % of all HAIs (Chenoweth and Saint 2011). Multidrug resistant microorganisms are able to colonize the inner and outer surfaces of indwelling or temporary catheters and to form polymicrobial biofilms (Frank et al. 2009) that persist on bladder epithelium, despite the removal of catheter, and resist antibiotic penetration (Blango and Mulvey 2010). The biofilm-forming pathogen most commonly implicated in urogenital infections is *E. coli* (Wang et al. 2010).

The involvement of fastidious anaerobic bacteria in many different types of urinary tract infections, including para- or peri-urethral cellulitis or abscess, acute and chronic urethritis, cystitis, acute and chronic prostatitis, pyelonephritis, renal abscess, and other infections, has been highlighted even if little attention has been paid so far (Brook 2004b). In a quite recent study, on 1,449 urine specimens examined both by culture and by PCR, the anaerobic bacteria detected only by using PCR (22.43 %) were *Bacteroides spp.*, pigmented *Prevotella spp.*, *Porphyromonas* sp., *F. magna*, *Peptostreptococcus vaginalis*, and *Bifidobacterium* spp. (Imirzalioglu et al. 2008).

Back in 1973, it was demonstrated that patients with indwelling urethral catheters had a high incidence of anaerobes recovered from urine (Alling et al. 1973) and later it was reported that patients with indwelling Foley catheters showed anaerobes along with aerobes and facultative organisms in urine samples (Sapico et al. 1976).

Nevertheless, even if strictly anaerobic bacteria have been found in the bladder urine of some patients with indwelling urethral catheters, no other specific studies have been published so far on their role in the initiation and perpetuation of CAUTIs and on their contribution in forming a polymicrobial biofilm on the urinary catheter surfaces.

### 6.7 Prosthetic Joint Infections

Infection processes, although uncommon, are the most serious complications occurring after prosthetic joint surgery. In fact, orthopaedic implants, surgically implanted into sterile areas of the body, can be colonized as a consequence of a transient sepsis, thus requiring additional surgery for revision arthroplasty (Sendi and Zimmerli 2011; Cobo and Del Pozo 2011). According to epidemiological data, prosthesis-related infections take place in 0.8–1.9 % of knee arthroplasties (Jämsen et al. 2009) and in 0.3–1.7 % of hip arthroplasties (Del Pozo and Patel 2009).

As increasingly reported in the recent years, single and multi-species biofilms are recognized as the main responsible for these implant-associated infections that are highly resistant to antibiotic treatment, due to poor penetration of antimicrobial molecules through the biofilm matrix, and to the host immune responses (McDowell and Patrick 2005; Song et al. 2013).

Most of the orthopaedic implants-associated infections are caused by staphylococci (about four out of five), particularly *CoNS* species (30–43 %).
and *S. aureus* (12–23 %), followed by Streptococci (9–10 %), Enterococci (3–7 %), gram negative bacilli (3–6 %), and anaerobes (2–4 %). Polymicrobial infections are observed in about 10–11 % (Zimmerli and Moser 2012).

However, one of the main problems encountered in determining the severity and the rate of infection is the difficulty to isolate biofilm-forming bacteria from prosthetic surfaces, especially anaerobes and microorganisms in viable but nonculturable state. This problem can be overcome with the use of molecular identification procedures, such as PCR combined with cloning, immunofluorescence microscopy (IFM) and FISH (Høgdall et al. 2010), with specific transport media for fastidious and robust aerobes and anaerobes (Tano and Melhus 2011) or with the placing the implants in an anaerobic jar directly after surgical removal (Tunney et al. 1998).

In this regard, Dempsey and co-workers have demonstrated that conventional identification techniques led to the detection of biofilm-forming bacteria on surfaces of the hip prosthesis in only the 22 % of cases compared to a detection rate of 72 % using molecular identification methods based on 16S rRNA. In the same study authors were able to identify also anaerobic species, such as *B. fragilis* (Dempsey et al. 2007).

Furthermore, molecular methods have also increased the sensitivity of *P. acnes* detection, thus becoming evident that many cases of ‘aseptic’ prosthesis loosening might due to *P. acnes* infections. In fact, the number of delayed joint prosthesis infections caused by this microorganism has been so far significantly underestimated (Tunney et al. 1999).

More recent studies have confirmed that *P. acnes* is an important cause of invasive infections related to prosthetic joint surgery, this anaerobe being isolated at a relative frequency comparable to many other pathogens (Lutz et al. 2005; Zeller et al. 2007; Zapperi et al. 2008; Portillo et al. 2013) and being able to form biofilm both in vitro and in vivo (Ramage et al. 2003; Bayston et al. 2007; Coenye et al. 2007; Tunney et al. 2007).

Even if the ability of *P. acnes* to form biofilm is now firmly established, the regulation of biofilm production and the differences in biofilm formation by diverse clinical strains are still poorly explored. Holmberg and co-workers examined a large collection of *P. acnes* isolates and showed that strains collected from deep infections related to foreign material produce more biofilm in vitro with respect to the isolates from skin of healthy individuals. This finding provides evidence that *P. acnes* biofilm production is affected by the isolation site, genes encoding biofilm components being subjected to environmental influences. This phenomenon is important for a better understanding of delayed joint prostheses infections caused by this microorganism (Holmberg et al. 2009).

It has been also demonstrated that the presence of human plasma in solution, or at the plastic surface, inhibits *P. acnes* biofilm formation, which could explain why it primarily infect the plasma-poor environments of joint prostheses (Levy et al. 2008; Holmberg et al. 2009).

The management of severe joint prostheses infections caused by *P. acnes* involves a combination of antimicrobial treatment and surgical intervention for the device removal. Intravenous penicillin G and ceftriaxone are the first choice for these serious infections, with vancomycin and daptomycin as alternatives, and amoxicillin, rifampicin, clindamycin, tetracycline, and levofloxacin for oral treatment (Portillo et al. 2013).

*Clostridium* spp., easily isolated from the human intestinal tract, has been also recognized as potential pathogen of prosthetic joint infections, penetrating trauma and hematogenous spread, a concomitant systemic infection being considered the most important source of infection. Although *Clostridium* spp. has been isolated in different orthopaedic infections, data about pathogenesis, natural history, and treatment of these infections are scarce (McCarthy and Stingemore 1999; Lazzarini et al. 2004).

A better understanding of biofilm formation mechanisms of *P. acnes* and *Clostridium* spp. and their role in the polymicrobial biofilm formation could help to set up innovative strategies to counteract delayed joint prostheses infections.
6.8 Bloodstream Infections

Central venous catheters (CVC) are among the most frequent causes of healthcare-associated bloodstream infections (Mermel et al. 2001; Zingg et al. 2009). Catheter-related bloodstream infections (CRBSIs) in ICU patients are associated with sepsis in the 28% of cases (Alberti et al. 2002), the intravascular portion of the device being rapidly coated, after the CVC insertion, by a rich layer of host-derived proteins that promotes adherence and biofilm formation of both blood-borne microbes and those introduced during the catheter insertion (Passerini et al. 1992). The biofilm remains in the tract also after catheter removal, rendering the patient susceptible to chronic establishment of biofilm and increasing the risk of continuous hematogenous bacterial spread (Donelli 2006).

The presence on the CVC surfaces of anaerobic species growing as biofilm, alone or within a polymicrobial biofilm, has been poorly investigated. In fact, the first evidence dates back to 1988 when Haslett and co-workers isolated Clostridium spp. and Propionibacterium spp. directly from indwelling central intravascular catheters (Haslett et al. 1988). After that, just few studies have demonstrated the presence of anaerobic species in microbial biofilms causing CRBSIs.

Although current guidelines for the management of CR-BSIs include Propionibacterium spp. as a potential infectious agent, P. acnes is rarely reported as cause of intravascular colonization or CR-BSI (O’Grady et al. 2002).

Martín-Rabadán and coworkers in 2008 have advanced the hypothesis that the low rate of catheter colonization and CR-BSI by Propionibacterium spp. reported in the medical literature is a consequence of an inappropriate laboratory detection methodology, the chances of detecting Propionibacterium bacteremia being reduced by including automatic detection of growth without terminal subcultures, reduction of incubation times, and elimination of anaerobic bottles. Authors demonstrated that P. acnes frequently colonize vascular catheter tips and suggested sequential aerobic-anaerobic processing as a simple procedure to analyze catheter tips by the roll-plate method (Martín-Rabadán et al. 2008).

Also a recent study for the detection of colonization and CR-BSI include Propionibacterium spp. as a potential cause, being isolated by anaerobic processing of catheter-cultures in the 8% of cases (Guembe et al. 2012).

The potential of the anaerobic biofilm former P. acnes as a cause of catheter-related bacteremia deserve further studies.

6.9 Conclusions

Most of the infections caused by anaerobes are considered opportunistic infections, arising from microorganisms of the normal flora that take advantage of generalized or localized defects in defence mechanisms to damage the host.

These anaerobic opportunistic pathogens are difficult to isolate and thus are frequently missed when clinical samples are cultured, their isolation requiring appropriate methods of collection and transport as well as cultivation of specimens in properly equipped clinical microbiology laboratories.

In fact, there is growing interest in a more accurate routine identification of anaerobes, for example by applying matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) or 16S rRNA gene sequencing (La Scola et al. 2011; Jamal et al. 2013).

Furthermore, even if highly virulent anaerobes should be considered for testing their antibiotic resistance as individual isolates (Brook et al. 2013), the slow growth, the often polymicrobial nature as well as the increasing antimicrobial resistance over time of these microorganisms, make not routinely performed the in vitro susceptibility testing (Nagy 2010).

Therefore, the treatment of these infections is mostly empirical and based on the administration of antimicrobial agents with known efficacy against anaerobes.

However, it’s important to take into account that the spectrum of antibiotic resistance among anaerobes is significantly changed during the last decades and, nowadays, it includes also carbapenems and nitroimidazoles. In fact, these
drugs, once considered universally active, now exhibit a variable efficacy depending on the geographical area. For this reason, the CLSI recommends periodic monitoring of resistance trends of clinically relevant anaerobes to select the best empirical antimicrobial therapy (Wybo et al. 2014).

Just to give an example, the best selection of antibacterial drugs against both facultative and strictly anaerobic bacteria in respiratory infections are β-lactams with β-lactamase inhibitors, clindamycin, cephemycins and carbapenems, since the rates of β-lactamase production are low for *Peptostreptococcus* spp. and *Fusobacterium* spp., while are high for *Prevotella* and *Bacteroides*; the drug resistance rates to ampicillin are high in *Prevotella* spp. and *Bacteroides* spp., while the rate to piperacillin is moderate in *Bacteroides*. By contrast, the drug resistance rates to combinations of these drugs, i.e., piperacillin and tazobactam (TAZ/PIPC), for all the most insidious anaerobic bacterial species, are low (Japanese Society of Chemotherapy Committee on guidelines for treatment of anaerobic infections 2011).

According to the whole findings reported in the last decades, the demonstrated ability to form biofilm of opportunistic anaerobic species and their possible role as causative agents of HAIs should alert even more clinicians and microbiologists on the need to search for anaerobes in clinical samples, when their presence can be reasonably assumed, and carefully verify their antibiotic susceptibility.

In fact, the desirable availability in clinical microbiological laboratories of appropriate facilities for isolation of anaerobes could add significant information on the possible contribution of anaerobic species to biofilm-based polymicrobial infections and, thus, drive the antimicrobial therapy in the right direction.

**Acknowledgments** The authors are indebted to Dr Paola Mastrantonio, Dr. Patrizia Spigaglia, Dr. Fabrizio Barbanti and Dr. Rita Cardines from the Istituto Superiore di Sanità, Rome, for their advices in cultivation, isolation and identification of anaerobes in clinical samples. The generous gift of *Propionibacterium acnes* clinical isolates by Professor Anna Maria Cuffini, University of Turin, is also gratefully acknowledged.

**References**


Blango MG, Mulvey MA (2010) Persistence of uropatho-


Su S, Hassett DJ (2012) Anaerobic *Pseudomonas aeruginosa* and other obligately anaerobic bacterial biofilms growing in the thick airway mucus of chronically infected cystic fibrosis patients: an emerging paradigm or “old hat”? Expert Opin Ther Targets 16:859–873


学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具