

ESCMID* guideline for the diagnosis and treatment of biofilm infections 2014

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Abstract

Biofilms cause chronic infections in tissues or by developing on the surfaces of medical devices. Biofilm infections persist despite both antibiotic therapy and the innate and adaptive defence mechanisms of the patient. Biofilm infections are characterized by persisting and progressive pathology due primarily to the inflammatory response surrounding the biofilm. For this reason, many biofilm infections may be difficult to diagnose and treat efficiently. It is the purpose of the guideline to bring the current knowledge of biofilm diagnosis and therapy to the attention of clinical microbiologists and infectious disease specialists. Selected hallmark biofilm infections in tissues (e.g. cystic fibrosis with chronic lung infection, patients with chronic wound infections) or associated with devices (e.g. orthopaedic alloplastic devices, endotracheal tubes, intravenous catheters, indwelling urinary catheters, tissue fillers) are the main focus of the guideline, but experience gained from the biofilm infections included in the guideline may inspire similar work in other biofilm infections. The clinical and laboratory parameters for diagnosing biofilm infections are outlined based on the patient's history, signs and symptoms, microscopic findings, culture-based or culture-independent diagnostic techniques and specific immune responses to identify microorganisms known to cause biofilm infections. First, recommendations are given for the collection of appropriate clinical samples, for reliable methods to specifically detect biofilms, for the evaluation of antibody responses to biofilms, for antibiotic susceptibility testing and for improvement of laboratory reports of biofilm findings in the clinical microbiology laboratory. Second, recommendations are given for the prevention and treatment of biofilm infections and for monitoring treatment effectiveness. Finally, suggestions for future research are given to improve diagnosis and treatment of biofilm infections.

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Introduction

Bacteria and fungi occur as individual, free-floating (planktonic) cells or clustered together in aggregates of cells (biofilms). A microbial biofilm is 'a structured consortium of microbial cells surrounded by a self-produced polymer matrix' [1]. In addition to microorganisms, components from the host, such as fibrin, platelets or immunoglobulins, may be integrated into the biofilm matrix. Both bacteria and fungi can cause biofilm infections and biofilms may consist of one microorganism or be polymicrobial [1–4]. Some biofilms adhere to natural or artificial surfaces in the host (including devices), while others may consist of aggregates associated with but not directly adherent to the surface (Fig. 1) [3,4]. Importantly, biofilms are characterized by physiological and biochemical gradients (nutrients, oxygen, growth rate, genetics) from the surface to the deeper layers of the aggregated structure. Biofilm-growing microorganisms express different specific properties compared with planktonically (= non-aggregated) growing microorganisms [4]. The observation of aggregated microbes surrounded by a self-produced matrix adhering to surfaces or located in tissues or secretions is as old as microbiology since both Leeuwenhoek and Pasteur described the phenomenon [5]. In environmental and technical microbiology, biofilms were already shown,

80–90 years ago, to be important for biofouling on submerged surfaces, e.g. ships. The concept of biofilm infections and their importance in medicine is, however, less than 40 years old and started by Høiby's observations of heaps of *Pseudomonas aeruginosa* cells in sputum and lung tissue from chronically infected cystic fibrosis (CF) patients and Jendresen's observations of acquired dental pellicles [5]. The term biofilm was introduced into medicine in 1985 by Costerton [5]. During the following decades it became obvious, that biofilm infections are widespread in medicine and odontology, and their importance is now generally accepted [5].

Biofilms typically cause chronic infections, which means that the infections persist despite apparently adequate antibiotic therapy and the host's innate and adaptive defence mechanisms. Chronic infections are characterized by persistent and progressing pathology, mainly due to the inflammatory response surrounding the biofilm [6]. This also means that persisting local inflammation is the only common feature of various biofilm infections, whereas other signs and symptoms depend on the impairment—if any—of the function of the organ/foreign body infected by a microbial biofilm [5,6]. The inflammatory cells dominating the persisting biofilm infection may be polymorphonuclear neutrophil leucocytes or mononuclear cells depending on whether the predominating immune response to

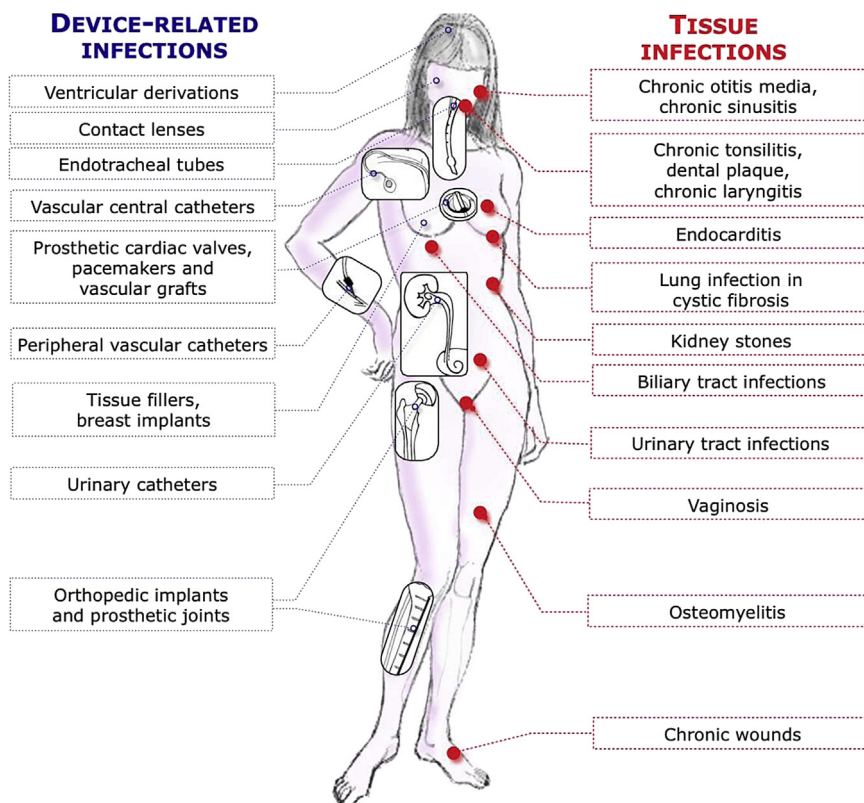


FIG. 1. Typical biofilm infections (3) (reproduced with permission).

the microbes of the biofilm is T helper type 2 (antibodies) or T helper type 1 (cell-mediated) polarized [5,8]. Many biofilm infections are difficult to diagnose and treat and currently no guidelines exist to help clinicians and clinical microbiologists with these infections.

Most microorganisms in the environment grow as biofilms in order to be protected against the hostile environment [7]. Traditionally, clinical microbiology laboratories have focused on culturing and testing planktonically (= non-aggregated) growing microorganisms and have reported the susceptibility to various antibiotics and antiseptics under planktonic growth conditions. Microorganisms in the biofilm mode of growth, however, cause chronic infections in tissues and on the surface of medical devices. The clinical implications of microorganisms growing as biofilms are that they may be more difficult to recover from clinical samples, and that they are physiologically much more

resistant to the effects of antibiotics and disinfectants [6]. Moreover, antibiotic therapy based on susceptibility testing of planktonic microorganisms may be associated with treatment failure or recurrence of the infection. It is the purpose of the guideline to bring the current knowledge of biofilm diagnosis and therapeutic practice to the attention of clinical microbiologists and infectious disease specialists.

The guideline addresses the diagnosis and treatment of biofilm infections. Such infections can be classified into those where biofilms are found in the tissue/mucus and not associated with foreign bodies and those where biofilms are adherent to foreign bodies [3] (Fig. 1). The first group includes CF patients with chronic lung infections (Figs. 2, 3) and patients with chronic wound infections (Fig. 4), where the biofilms are found in the tissue and also in sputum of patients with CF [8–10]. The second group includes infections related to biofilms colonizing

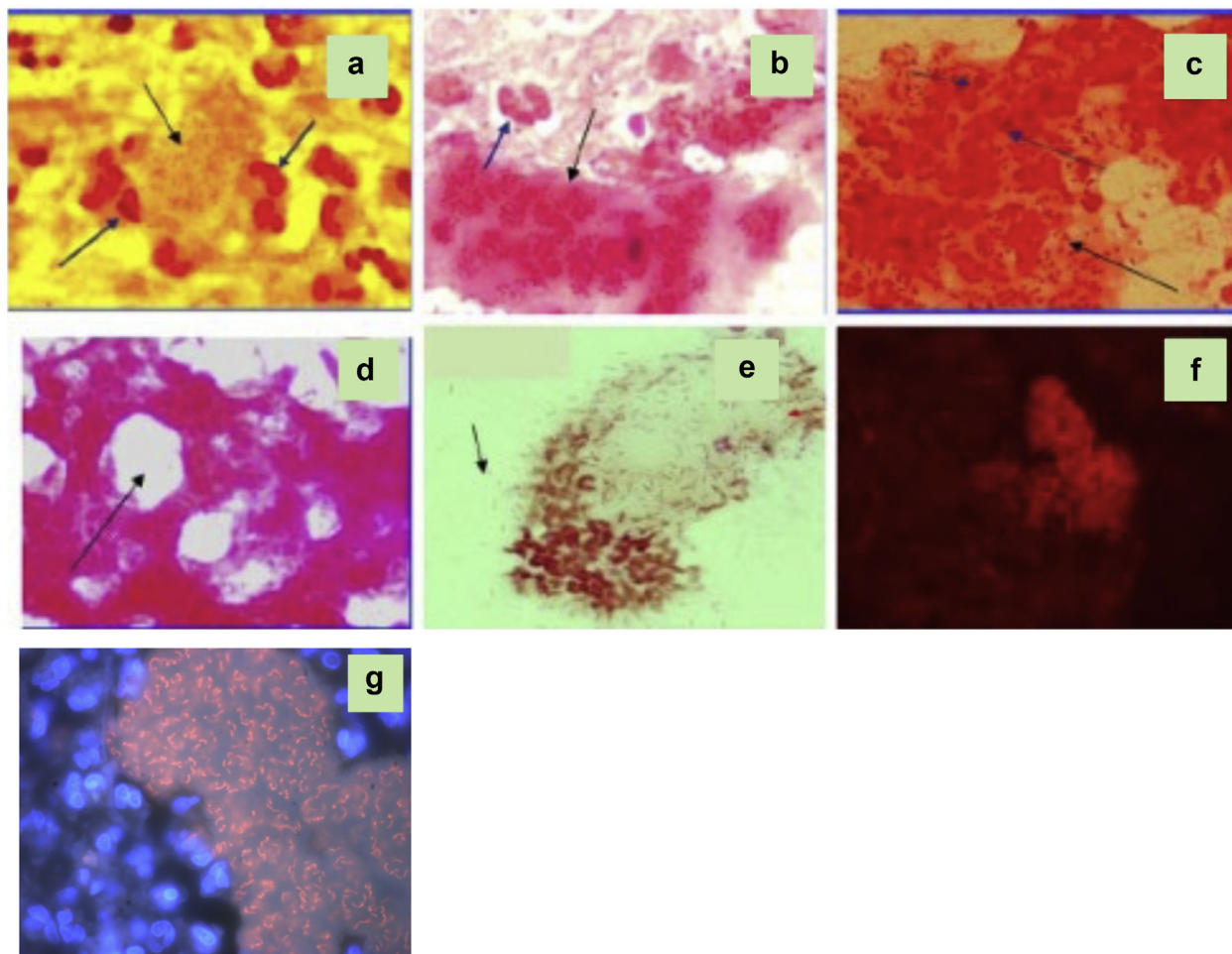


FIG. 2. Biofilm causing tissue infection. Biofilms of *P. aeruginosa* from sputum of cystic fibrosis patients. Gram-staining (a-e), PNA-FISH staining with a *P. aeruginosa* specific probe (f, g). The bacteria and the matrix are visible. A diversity of shapes of the biofilms are seen, a: with surrounding polymorphonuclear leukocytes (arrows), b & c: with a few leukocytes within the alginate matrix, d: with channel-like holes (arrow), e: with liberated planktonic bacteria (arrow). Magnification $\times 1000$ [8] (reproduced with permission).

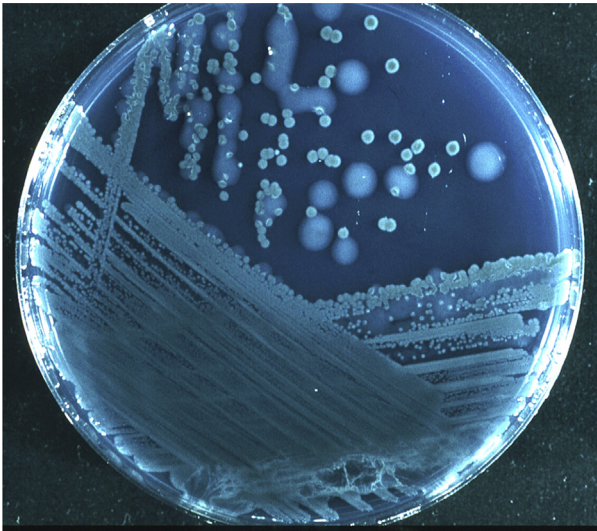


FIG. 3. *P. aeruginosa* from sputum of a cystic fibrosis patient. Mucoïd (large) and nonmucoïd (small) colonies. The mucoïd variant overproduces alginate which is the matrix in the *P. aeruginosa* biofilm in the respiratory tract of cystic fibrosis patients. Mucoïd colonies are only found in patients with chronic biofilm infection and alginate from mucoïd colonies is therefore a biofilm-specific antigen.

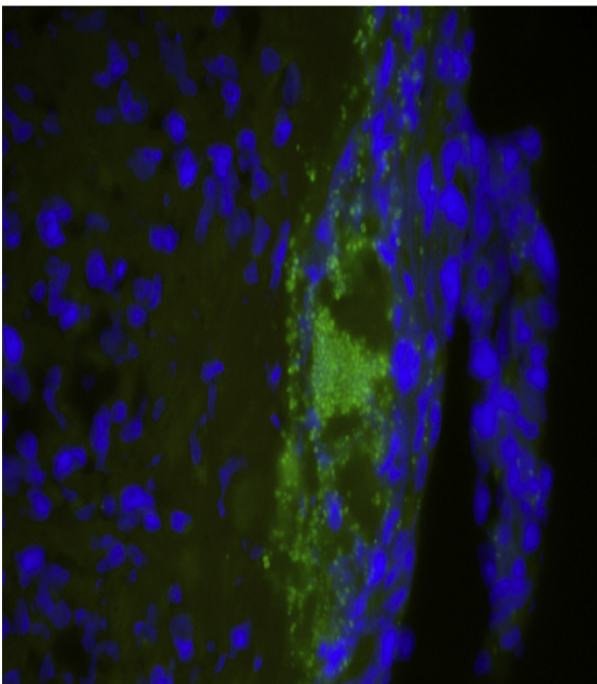


FIG. 4. Biofilm causing tissue infection. Biofilm of *S. aureus* in a chronic wound. The bacteria were identified by a specific PNA-FISH probe (green stain) and the host cells visualised by DAPI (blue stain). Magnification $\times 1000$ [9] (reproduced with permission).

devices implanted inside the body or forming a connection between an inner or outer surface of the body where a normal microbial flora is present and a sterile anatomical compartment inside the body (Figs. 1, 5–9; Table 1). This group includes infections associated with orthopaedic alloplastic devices [11], endotracheal tubes [12–15], intravenous catheters [16], indwelling urinary catheters or urethral stents [17,18] and tissue fillers [19,20] including, but not restricted to, breast implants [21]. Many other biofilm infections are the subject of existing guidelines (e.g. endocarditis, otitis media, chronic sinusitis, biliary stents, shunts, contact lenses, voice prostheses, dialysis catheters, dental, intrauterine devices, artificial hearts, prosthetic valves). However, the present guideline will focus on the biofilm infections outlined above. The target professionals are clinical microbiologists and infectious disease specialists involved in diagnosis and treatment of biofilm infections.

The overall burden of biofilm infections is significant [22]. For example, among the >60 000 CF patients in developed western countries, nearly 80% will develop a chronic biofilm lung (and paranasal sinus) infection. For patients with chronic wound infections (1–2% of western populations) more than 60% have been shown to involve biofilms. For all patients with orthopaedic alloplastic device, 0.5–2% will develop an infection within the first two postoperative years [23–25]. Additionally, among patients with intravenous catheters, 5 per 1000 catheter days in the intensive care unit will develop catheter-related bloodstream infections (CR-BSI). The development of biofilm on the surface of endotracheal tubes (ETT) is related to the development of ventilator-associated pneumonia (VAP), which occurs in 9–27% of all intubated patients [26]. The rates for patients with indwelling urinary catheters are even higher because more than 50% of inserted catheters become colonized within the first 10–14 days of insertion [27,28] and for patients with tissue fillers where 1–2% experience adverse events of which almost all arise from biofilm infections. Post-mastectomy tissue-expander infections occur in 2–24% of patients [29].

For many of the above infections—with the possible exception of CF—there are controversies and uncertainties concerning both the diagnosis of biofilm infections and treatment or prophylactic approaches using antibiotics. These issues have been discussed in depth in all six American Society for Microbiology and three European Society for Clinical Microbiology and Infectious Diseases (ESCMID) conferences on biofilm infections from 1996 to 2013. These conferences and discussions about biofilm infections, as well as numerous textbooks, emphasize that in clinical settings the diagnosis and treatment of biofilm infections is inconsistent. This calls attention to the need for the development of evidence-based guidelines.

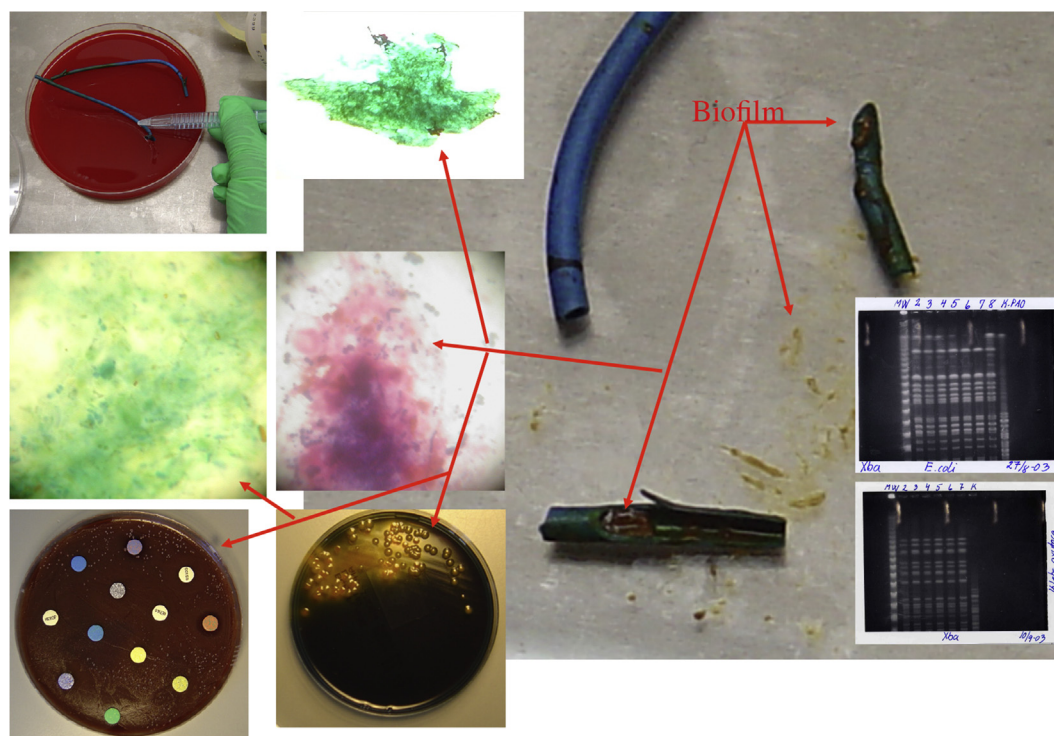


FIG. 5. Biofilm causing device-related infection and as the focus for systemic infection. A biofilm is located on a pancreatic-biliary stent from a 57 years old man. The biofilm was the focus of recurrent sepsis with *E. coli* (July 21-2003) where he was treated with adequate (planktonic susceptibility testing) antibiotics for 5 days and with the same *E. coli* strain and *K. oxytoca* (August 12, 2003) where he succumbed in spite of antibiotic treatment. Autopsy on August 14, 2003 showed growth from the biofilm of *E. coli*, *K. oxytoca* (same Pulsed Field Gel Electrophoresis (PFGE) type as isolated from the blood previously) and *E. faecium* which was not isolated from the blood. The figure with red arrows shows the stent held above a 9 cm petri dish with blood agar (upper left), the stent which has been opened (right), Methylene- and Gram-stained smears (magnification x 1000) and results of culture and susceptibility testing and PFGE. (111)(Reproduced with permission).

For some of the biofilm infections mentioned above, e.g. CF [30–34], intravenous catheters [16], indwelling urinary catheters or urethral stents [17,18,35,36] and periprosthetic joint infections [37,38], there is sufficient scientific evidence of good quality to allow development of a guideline document. For others such as chronic wounds [9,39,40] and tissue fillers [19], there is good quality evidence for diagnostic approaches but probably not yet for therapy. As for VAP associated with ETT biofilm, in all available studies [12,14,41,42], the ETT biofilm has been evaluated following extubation. Hence, no reliable methods are currently available to detect ETT biofilm while the patient remains on invasive mechanical ventilation. Conversely, a few preventive and therapeutic strategies to reduce ETT biofilm formation and VAP have been tested in clinical settings [43–46].

The expert panel has completed a systematic review of the literature within the specific areas outlined above and prepared this document based on the systematic reviews including key questions concerning diagnosis, prophylaxis and treatment of

biofilm infections and has evaluated the strength of the recommendations and quality of evidence (Tables 2 and 3).

Diagnosis of Biofilm Infections

Generally: Biofilms are small *in vivo*, in tissues 4–200 μm , on foreign bodies 5–1200 μm (Table 4) [10]. Therefore, the search for biofilms in clinical samples may be difficult and time consuming, and may result in false-negative results if the samples are not representative of the focus of the biofilm infection. Biofilms can be released from artificial surfaces by sonication of fluids containing the sample [47].

Q1-1 Which type of samples should be sent to the clinical microbiological laboratory to detect biofilm infections?

CF with chronic lung infections. A major problem in this type of infection is contamination of lower respiratory secretions with

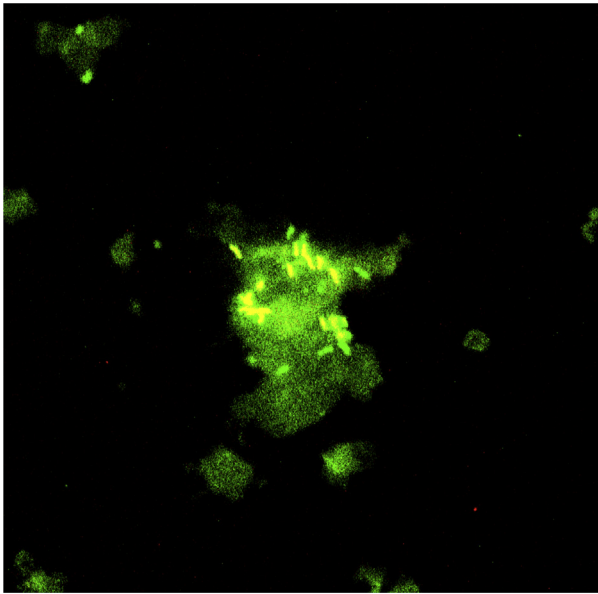


FIG. 6. Biofilm causing device-related infection. PNA-FISH micrograph of catheter biofilm. Material scraped from a urinary catheter was hybridized with a universal bacterial probe (UUBmix) and an *Enterobacteriaceae* probe. Single cells and clusters of enterobacteria are visible in the biofilm. The large green background indicates unspecific binding of the EUBmix probe to the biofilm. *E. faecalis* and *E. coli* were isolated from the catheter tip by culture (82)(reproduced with permission).

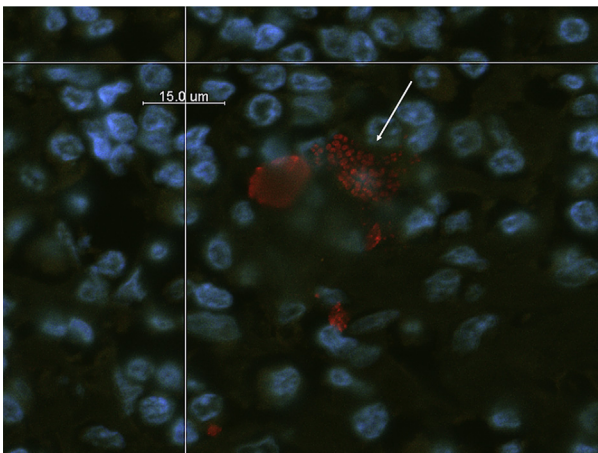


FIG. 7. Biofilm causing device-related infection. Three-dimensional confocal laser scanning microscopy of a biopsy from a grade 3 reaction following gel injection into the cheek 2 years previously. A PNA-FISH universal bacterial probe was used to visualize aggregates of bacteria (small red micropheres; arrows). The large blue dots represent DAPI nuclear counterstain of the predominating mononuclear cell types in these lesions. *S. epidermidis* had been identified by 16S rRNA gene sequencing. Magnification $\times 1000$ (19)(reproduced with permission).

the normal oropharyngeal flora, particularly since members of the normal flora (e.g. *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Moraxella catarrhalis*) are common lung pathogens in CF [48]. Representative material originating from lower airways (sputum, protected samples through bronchoalveolar lavage, endolaryngeal suction (from small children), induced sputum) should be sent to the clinical microbiological laboratory (CML) [30–34] (All).

Patients with chronic wound infection. Biopsy tissues are considered the most reliable samples to reveal biofilm in wounds. The use of swabs to collect biofilm samples from the wound surface is considered an inadequate method (DII), due to contamination from the skin flora, the strong adherence of biofilm to the host epithelium and the growth of anaerobes in the deep tissues. If a moderate to severe soft tissue infection is suspected and a wound is present, a soft tissue sample from the base of the debrided wound should be examined. If this cannot be obtained, a superficial swab may provide useful information on the choice of antibiotic therapy [49,50] (All).

Patients with infections related to an orthopaedic alloplastic device (e.g. an artificial joint). If an orthopaedic implant-associated infection is suspected, synovial fluid should be sampled for cell counts and microbiological work-up. If the results suggest or confirm infection, debridement surgery is indicated (AIII). Intraoperative sampling includes biopsies from representative peri-implant tissue and removal of the device/prosthesis or modular parts of it (e.g. inlay, screws). This foreign material should be submitted for sonication to liberate the biofilm before being cultured in the CML [51] (BII). The Clinical Practice Guidelines on Diagnosis and Management of Prosthetic Joint Infection by the Infectious Diseases Society of America [37] recommend sampling of three to six biopsies. The presence of at least two positive culture specimens with an identical microorganism confirms periprosthetic joint infection [52–56]. The biopsies should be as large as possible (up to 1 cm³) and labelled and paired with the culture specimens to differentiate contamination from infection [57–60] (AIII).

Patients with endotracheal tube biofilm, VAP. There is a lack of validated methods to monitor biofilm formation within the ETT and to distinctly recognize its role in the development of VAP. First, in mechanically ventilated patients who develop VAP, biofilm within the ETT can be either the primary source of the infection or merely a concomitant colonized site. Second, respiratory secretions often accrue within the ETT; as a result, biofilm and respiratory secretions constitute a complex mixture that is difficult to characterize. The presence of sessile pathogens adherent to the ETT surface may be overestimated, because of the vast concentration of planktonic pathogens

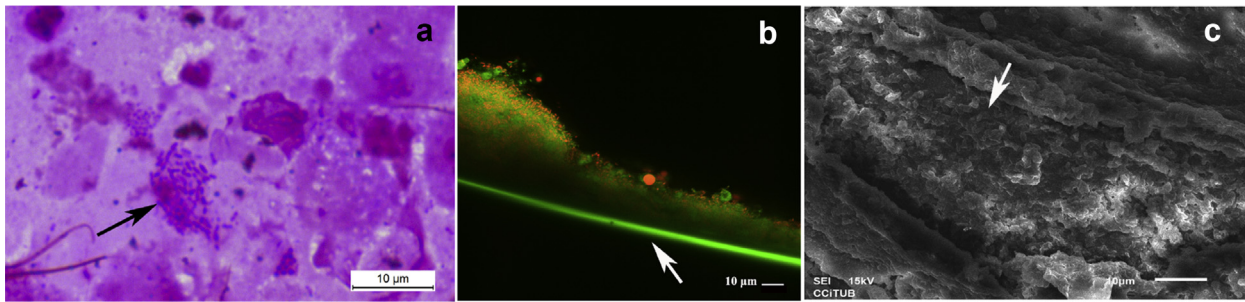


FIG. 8. Microscopy studies of the endotracheal tube of a pig invasively mechanically ventilated for 72 hours, following oropharyngeal challenge with *Pseudomonas aeruginosa* (Li Bassi et al. *Anesthesiology*, accepted, 2013). a: Light microscopy of biofilm and respiratory secretions retrieved from the inner surface of the endotracheal tube (magnification x1000, oil immersion objective lens). Biofilm/secretions were spread on a glass slide, and stained with Congo Red and Crystal Violet for light microscopic studies [63]. The black arrow indicates an aggregate of rod-shaped bacteria; microorganisms stain as purple, and the biofilm exopolysaccharide stains as pink. b: Confocal laser scanning micrograph of the internal surface of the endotracheal tube (magnification x250). The sample was stained with BacLight Live/Dead (Invitrogen, Barcelona, Spain). The white arrow depicts the endotracheal tube outer surface. A fully mature biofilm adherent to the endotracheal tube is shown and rod-shaped bacteria are embedded within the biofilm matrix. c: Scanning electron micrograph frontal-view of the internal surface of the endotracheal tube (magnification x1500) (Fernández-Barat et al. *Crit Care Med* 2012; 40: 2385-95)(Berra et al. *Anesthesiology* 2004; 100: 1446-56). Note presence of stage IV biofilm, characterized by multiple rodshaped bacteria embedded within an extracellular polymeric substance, as depicted by the white arrow. (Micrographs kindly provided by Laia Fernandez-Barat and Eli Aguilera Xiol, Hospital Clinic-CIBERES, Barcelona, Spain.)

colonizing the airway secretions. Hence laboratory and clinical studies [11,14] have found matching pathogens in both the ETT biofilm and secretions accrued within the airways/ETT in 56–70% of patients with VAP. A clinical guideline on the diagnosis of VAP [26] recommends that samples of lower respiratory tract secretions should be obtained and cultured from all patients with clinical suspicion of VAP, before change of antibiotic therapy, to identify the aetiology of VAP (AIII). Respiratory secretions can be obtained through endotracheal aspirate, bronchoalveolar lavage, or protected specimen brush

depending on the situation and possibilities. In addition to these sampling procedures, in patients with clinical suspicion of ETT biofilm VAP, mucus from within the ETT can be aspirated and cultured to identify ETT pathogens in ETT biofilms that may have caused VAP (BII). This is not necessary for the diagnosis of the aetiology of VAP but may lead to a decision to replace the ETT. Devices serving to remove secretions and biofilm from the inner lumen of ETT, i.e. the Mucus Shaver (described in Q2-2) [43,61,62], can be employed to dislodge biofilm and identify the aetiology of ETT biofilm VAP (BIII). Mucus and biofilm retrieved from the inner surface of the ETT can be processed in the CML for microscopic examination of the presence of bacterial aggregates and biofilm on the ETT luminal

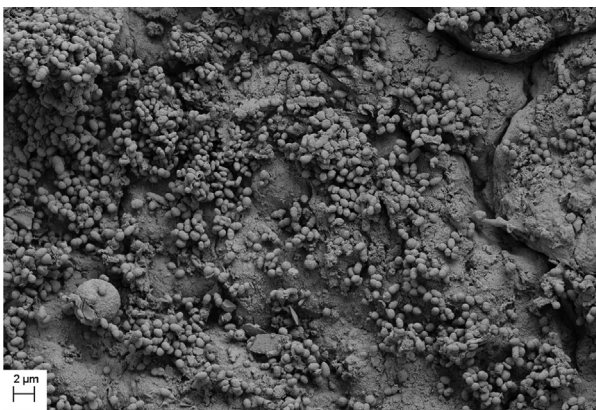


FIG. 9. Field emission scanning electron micrograph of a polymicrobial biofilm developed in the lumen of a Foley catheter removed from a patient affected by a catheter-associated urinary tract infection. The species identified by culture methods were *Acinetobacter baumannii*, *Enterococcus faecalis* and *Escherichia coli* (G. Donelli).

TABLE 1. Natural and pathogenic biofilms on human tissue and foreign bodies

Organ/anatomic compartment A with normal flora (microbiome)	Connection [may be via a foreign body]	Organ/anatomic compartment B without normal flora (microbiome)
Skin	->	Blood, peritoneum
Pharynx	->	Bronchi, lungs
Duodenum	->	Bile tract, pancreas
Urethra	->	Bladder
Vagina	->	Uterus
Air in operating room, skin flora ^a	->	Alloplastic material, neurosurgical shunt
No symptoms	->	Pathology

Reproduced from ref. [1] with permission.
^aMost frequently coagulase negative staphylococci and *Propionibacteria*, which exist as biofilms on detached epidermal cells.

TABLE 2. Definition of strength and quality of recommendations [196]**Strength of recommendation:**

Grade A: ESCMID strongly supports a recommendation for use

Grade B: ESCMID moderately supports a recommendation for use

Grade C: ESCMID marginally supports a recommendation for use

Grade D: ESCMID supports a recommendation against use

Quality of evidence:

Level I: Evidence from at least one properly designed randomized controlled trial.

Level II^a: Evidence from at least one well-designed clinical trial, without randomization; from cohort or case-control analytic studies (preferably from more than one centre); from multiple time series; or from dramatic results of uncontrolled experiments.

Level III: Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies.

^aAdded index:

Meta-analysis or systematic review of randomized controlled trials.

Transferred evidence, that is, results from different patient cohorts, or similar immune-status situation.

Comparator group is a historical control.

Uncontrolled trial.

Published abstract (presented at an international symposium or meeting).

wall (Fig. 8) [63,64] (BIII). After extubation, the ETT inner surface can also be processed in the CML for microscopic examination of the presence of biofilms [43,64–66] (Fig. 8) (BIII).

Patients with vascular catheters. If the catheter is removed, the catheter tip (3 to 4 cm distal) should be sent to the CML for quantitative or semi-quantitative culture [16,67,68] (All). In cases of totally implantable venous access port-related infection (e.g. Port-a-Cath®), also send the reservoir and/or the port to the CML [69,70] (All). In cases of signs of local infection (tunnel or port-pocket infection), send aseptically removed material

surrounding the port or tunneled catheter, such as purulent fluid or necrotic skin, ideally during the surgical procedure, to reduce the risk of false-positive results [71,72]. Superficial swabs of skin and catheter hubs are associated with a high negative predictive value for short-term as well as long-term catheters [73,74] (BII).

If catheter-related infection is suspected and the catheter is still *in situ*, send paired blood cultures from the vascular catheter and peripheral blood taken simultaneously. Two types of blood cultures can be used: qualitative blood cultures with data of the time to positivity (TTP) [73,75–77] (All) and quantitative blood cultures [73,78] (All). These two methods are

TABLE 3. General features of clinical and laboratory indications for diagnosis of biofilm infections^a

- Clinical signs of infection e.g. the classical but frequently low-grade inflammatory reactions tumor, rubor, dolor, loss of function and sometimes low-grade fever
- Medical history of biofilm-predisposing condition (e.g. implanted medical device, cystic fibrosis)
- Persisting infection lasting >7 days (this is unspecific, and other reasons are frequent such as resistance to the antibiotics used)
- Failure of antibiotic treatment and recurrence of the infection (particularly if evidence is provided that the same organism is responsible on multiple time points)—typing of the pathogen
- Documented evidence/history of antibiotic failure
- Evidence of systemic signs and symptoms of infection that resolve with antibiotic therapy, only to recur after therapy has ceased.

Microbiological diagnostics:

- Microscopic evidence from fluid/tissue samples obtained from the focus of the suspected infection
 - Microscopy revealing the presence of microbial aggregates and biofilm structure (smear or fluid sample, but ideally from tissue sample if possible)
 - Microscopy revealing evidence of microbial aggregates co-localized with inflammatory cells
 - Microbiological evidence of aggregated microorganisms consistent with infectious aetiology
- Positive culture/non-culture-based techniques (PCR) of fluid or tissue sample
 - Culture-based identification of microbial pathogens (MALDI-TOF)
 - Presence of mucoid colonies or small colony variants of *P. aeruginosa* in culture positive samples—which may indicate antibiotic recalcitrance)
 - PCR, quantitative PCR or multiplex PCR positive results for pathogen associated with infection (e.g. *Staphylococcus aureus* with implant, *Pseudomonas aeruginosa* with cystic fibrosis)
 - fluorescence in situ hybridization positive results for known pathogen showing aggregated microorganisms
 - Non-culture-based identification of microbial pathogen (pyrosequencing, next-generation sequencing).

- Specific immune response to identified microorganism—(e.g. *P. aeruginosa* antigens in cystic fibrosis patients) if the biofilm infection has been present for more than 2 weeks.

Modified from ref. [1].

^aSpecific features depend on possible impairment of the function of the organ/foreign body involved.

considered equal, but TTP is the most convenient in the routine CML because the TTP is recorded by modern blood culture systems.

Patients with indwelling urinary catheters or urethral stents. The simplest way to reveal catheter-associated urinary tract infections is to collect urine samples from the catheter (CI), although this method can lead to false-negative results in biofilm-positive microorganisms in 50–64% [36,79,80].

Removed catheters can also be analysed in the CML by microscopy and by cultivation or culture-independent techniques, preferably after sonication to investigate microbial diversity.

TABLE 4. Biofilm size in chronic infections in otherwise sterile parts of the human body^a (See also Table 3)

Biofilm demonstrated in	Visualization method	Approximate diameter	Reference
Lung infections (CF)	Light microscopy	~4–8 µm	[197]
	Light microscopy	~5–100 µm	[198]
	FISH	~5–100 µm	[8]
	FISH	~5–50 µm	[199]
Chronic wounds	FISH	~5–200 µm	[40]
	FISH	~5–100 µm	[9]
	Light and electron microscopy	~35–55 µm	[39]
Soft tissue fillers Otitis media	FISH	~5–25 µm	[20]
	FISH	~15–25 µm	[200]
	FISH	~10–80 µm	[201]
	FISH	~4–40 µm	[202]
Implant-associated	Electron microscopy	~500 µm	[203]
	FISH	~50 µm	[204]
	Electron microscopy	~5–15 µm	[205]
Catheter- and shunt-associated	FISH	~5–30 µm	[206]
	Electron microscopy	~5–1000 µm	[207]
	Electron microscopy	~20–500 µm	[208]
	Fluorescence microscopy	~20–1200 µm	[209]
Chronic osteomyelitis	FISH and electron microscopy	>1000 µm	[210]
	Electron microscopy	~25 µm	[211]
	Electron microscopy	~25 µm	[212]
Chronic rhinosinusitis	Light and electron microscopy	~5–50 µm	[213]
	Electron microscopy	~5–30 µm	[214]
	Fluorescence microscopy	~5–20 µm	[215]
Contact lenses	Electron microscopy	~50–100 µm	[216]

Abbreviations: CF, cystic fibrosis; FISH, fluorescence *in situ* hybridization.

^aThe biofilm aggregate size was estimated by measuring the longest diameter or length directly on the micrograph images in the source articles. (adapted from ref. [10]).

TABLE 5. Diagnostic use of three different anti-*Pseudomonas* antibody methods to detect chronic *Pseudomonas aeruginosa* biofilm infection in Scandinavian cystic fibrosis patients [103]

	Crossed immune-electrophoresis (95% CI)	<i>Pseudomonas</i> -CF-IgG ELISA (95% CI)	Exotoxin A ELISA (95% CI)
Specificity	89% (86–92)	83% (78–87)	89% (85–92)
Sensitivity	96% (93–98)	97% (94–99)	93% (89–96)
Positive predictive value	87% (82–90)	80% (75–84)	86% (81–90)
Negative predictive value	97% (95–99)	98% (95–99)	95% (92–97)
Positive predictive value after patients with other Gram-negative infections were excluded	93% (89–95)	85% (80–89)	88% (84–92)
Negative predictive value after patients with other Gram-negative infections were excluded	97% (95–99)	97% (94–99)	95% (92–97)

This approach ensures the detection of biofilm infection because of isolation of only the adherent bacteria [36,80–85] (All).

Patients with infections connected to tissue fillers e.g. breast implants. Removed material and contiguous material (BIII).

Q1-2 Which methods should be used in the CML to detect biofilms in the samples?

Detection of biofilms in the samples requires that microscopy should show evidence of an infective process, such as the presence of leucocytes, and that the microorganisms present are demonstrated to be microbial aggregates embedded in an apparently self-produced matrix distinct from the surrounding tissue or secretion. Microscopic analysis can be done using routine light microscopy and routine staining methods including Gram stain, which stains both tissue or mucus and the inflammatory cells, bacteria and the biofilm matrix [8] (All). Techniques such as confocal laser scanning microscopy and scanning electron microscopy are the most appropriate to reveal biofilms in biopsies but they are not available for routine diagnostic work in CML [86] (BIII).

Specific microscopic identification of the biofilm microorganisms in samples (biopsies or swabs) can be done by means of species-specific fluorescence *in situ* hybridization probes and fluorescence microscopy [8] (All), whereas conventional culture methods or culture-independent methods based on PCR techniques (16S rRNA gene amplification, denaturant gradient gel electrophoresis, bacterial tag-encoded FLX amplicon pyrosequencing) [25,59] cannot discriminate between planktonic and biofilm-growing bacteria [82,83–86].

Furthermore, in clinical samples planktonic microorganisms are released from biofilms and microorganisms may therefore

be found in both forms. Release of microorganisms from biofilms may be achieved by imprint (and visualized by Gram stain), rolling (the Maki method), and semi-quantitative reporting of the number of colony-forming units (CFU) obtained, or by scraping, whirly-mixing, vortexing and/or sonication (Brun–Buisson method) [57,80,87–89]. Crushing or tissue homogenization may be necessary if the biofilm is situated on the surface of a foreign body or in a bone or a cardiac valve (All). Some microorganisms in biofilms may be viable but non-culturable [90] when using routine media. In that case culture-independent methods should be used [91,92] (BII). In areas of the body where a normal flora (microbiome) is present (in the mouth, pharynx, gut, skin) (Table 1), special precautions—such as washing or irrigating the area—should be taken to minimize contamination from the normal flora. Since culture or culture-independent techniques (e.g. PCR) cannot distinguish between biofilm-growing and planktonic microorganisms, the identification of biofilms relies on microscopic detection of microbial aggregates located in a matrix. It should be noted that low or non-pathogenic members of the normal flora may be important biofilm producers (e.g. *Staphylococcus epidermidis* biofilms on intravenous lines or on orthopaedic alloplastic material). Ideally the results of microscopy and culture should be quantitative or semi-quantitative and organisms should be identified to the species level to help discriminate between contaminants from the normal flora and infecting microorganisms, regardless of whether they have previously been associated with biofilm infections.

Practice points. In the case of tubes, urinary tract catheters and vascular catheters, (intravenous lines) biofilms may be located on both outer and inner surfaces.

For diagnosis of catheter-related infection. Microscopy of Gram-stained imprints from, for example, tips can be used as rapid detection of biofilm infections [16] (All).

The culture of the catheter tip can be made with a quantitative or a semi-quantitative method. For quantitative culture, the tip is sonicated or vortexed in a 1-mL solution with a significant threshold of $\geq 10^3$ CFU/mL to distinguish between colonization and contamination (Brun–Buisson method) (All). For the semi-quantitative method, the catheter tip is rolled on an agar plate (Roll-plate method, also called the Maki method). The threshold is ≥ 15 CFU [68] (All). A meta-analysis published in 1997 seemed to favour the quantitative method [93] but a more recent paper stated that they could be considered equal for the diagnosis of tunnelled catheter-related infections [94]. Current Infectious Diseases Society of America guidelines consider that both methods can be used [16] and we came to the same conclusion (All).

For the diagnosis of catheter-related bloodstream infections. Paired blood cultures from a catheter and a peripheral vein with one of the two following differential criteria indicate biofilm infection: 1) TTP: ≥ 2 h earlier growth in the bottle with blood drawn from central venous catheter (CVC) [73,75,76] (All); 2) quantitative blood culture with various thresholds published so far [73,78] (All). Infectious Diseases Society of America guidelines propose the following threshold: a three-fold greater colony count in the blood drawn from CVC than in blood drawn from peripheral vein (All) [16].

In case of totally implantable venous access port-related infection (e.g. Port-a-Cath®). Peripheral blood culture associated with a positive culture of the catheter tip (*in situ* located in the bloodstream) or the subcutaneous septum (to be penetrated by needles for injection of drugs into the catheter) [69,70] (All). There is no consensus regarding methods to culture the port reservoir or septum. The following methods have been proposed: an adapted Brun–Buisson method of the septum after removal, the sampling of any macroscopic debris or clots present after septum removal or the swabbing of the internal surface of the port [72,74] (BIII). Some authors also propose the injection of sterile saline inside the port to recover fluid, which is subsequently cultured [95]. No data are available to say which method is more accurate. Recently, a paper suggested that the best strategy was to combine catheter tip culture, port sonication fluid and internal surface biofilm cultures [96].

Candida biofilms. Traditional techniques require device removal followed by culture or microscopy of a catheter segment. Catheter-sparing diagnostic tests include paired quantitative blood cultures, differential time to positivity of paired blood culture, catheter-drawn quantitative blood cultures, acridine orange leucocyte cytospin with or without Gram stain [97]. These *in situ* techniques avoid catheter removal strictly for diagnostic purposes [16,98]. Diagnosis of *Candida* biofilm (Fig. 10) using microbiological techniques poses major obstacles. Compared with many bacterial pathogens, blood cultures are not consistently positive for *Candida*, even in the case of systemic disease and previous antifungal therapy may also significantly impact the sensitivity of microbiological techniques, [99]. Furthermore, paired blood cultures have been poorly studied for fungal pathogens and are less accurate than for bacterial infection [99]. Sonication-vortexing recovers significantly more biofilm *Candida* CFU than brushing [99,100]. TTP of *Candida* species in culture bottles of peripheral blood may be a useful tool in the evaluation of patients with candidaemia who have an indwelling CVC, since TTP was shorter for definite catheter-related candidaemia (17.3 ± 2 h) than for candidaemia from other sources (38.2 ± 3 h). A TTP cut-off of

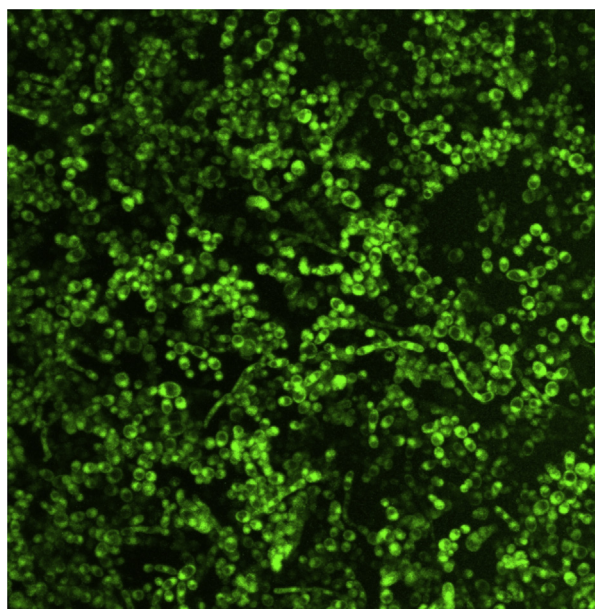


FIG. 10. Confocal laser scanning microscopy of a green-fluorescent-protein-tagged *Candida albicans* biofilm. Magnification $\times 1000$ (C. Imbert).

30 h was 100% sensitive and 51.4% specific for catheter-related candidaemia. So TTP in peripheral blood may be a sensitive, although non-specific, marker for catheter-related candidaemia and TTP of more than 30 h may help to exclude an intravascular catheter as the possible source of candidaemia (CIII) [101,102]. The number of positive peripheral blood cultures also seems to be a promising diagnostic tool to diagnose catheter-related candidaemia without directly removing the catheter [99].

In case of biofilm urinary tract infections in patients with indwelling urinary catheters or stents. Freshly obtained urine from the bladder should be processed as in non-catheterized patients bearing in mind that this procedure gives more than 50% false-negative results in these patients. Examination of removed catheters or stents is necessary for detection of biofilm infection; however, the routine examination of removed urinary catheters or stents is not recommended.

Biofilm-specific microbial phenotypes. These have rarely been described with the exception of mucoid (and maybe small colony variant) *P. aeruginosa* in CF (Fig. 3) [8,103]. This mucoid, slimy component is the polysaccharide alginate; however, no validated commercial methods to detect alginate in samples (e.g. sputum) are currently available. We recommend that growth of mucoid *P. aeruginosa* from clinical samples indicates the presence of biofilm infection and the mucoid phenotype should therefore be reported to the clinicians [8,103] (AI).

Q1-3 Are measurements of antibodies or inflammatory markers of any value to detect biofilm infections?

Significantly elevated levels of IgG antibodies against crude or purified antigens from *P. aeruginosa* antigens measured by various methods including ELISA, these elevated levels are diagnostic for *P. aeruginosa* biofilm infections in CF patients and such tests are commercially available and have been validated (Table 5) [103] and are recommended for detection of *P. aeruginosa* biofilm infection in CF patients (AI). Likewise, significantly elevated antibodies against antigens from other bacteria causing biofilm infections in CF patients (e.g. *Burkholderia cepacia* complex, *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*) have been reported, but no commercial tests are available. Some of the *P. aeruginosa* antibody tests have also been used in non-CF patients with chronic *P. aeruginosa* infections caused by mucoid phenotypes that also show significantly elevated antibody responses. IgM antibody response against biofilm-specific polysaccharide antigen in *S. aureus* and *S. epidermidis* alloplastic-related infections has also been reported [104,105] and can be recommended (BII). An elevated IgG and especially secretory IgA antibody response [106] with simultaneous negative cultures may encourage a search for hidden foci (e.g. paranasal sinuses). This is done by means of further samplings by more invasive techniques (e.g. bronchoalveolar lavage or nasal irrigation) and use of culture-independent techniques (e.g. 16S rRNA gene PCR) to detect microorganisms suppressed/killed by ongoing antibiotic therapy [107] and this is recommended (All). There are however, no widely available antibody measurement methods or inflammatory markers specific for biofilm infections.

Inflammatory markers. The non-specific inflammatory markers (C-reactive protein, procalcitonin, erythrocyte sedimentation rate, white blood cells), or various cytokines cannot distinguish between infections caused by planktonic cells and biofilm infections (DIII) [108,109].

Q1-4 How should the CML report to the clinician that biofilms have been detected?

Since an antibiotic treatment regimen for acute infections may fail in the case of biofilm infections and since foreign bodies associated with biofilm infections may have to be removed/replaced or the infection suppressed by antimicrobial treatment, it is important that the CML reports include whether microbial biofilms have been detected in patients' samples. It is important to note that identification of the presence of biofilms may be time consuming and needs to take into account the clinical condition of the patient so any clinician suspecting an infection due to a biofilm should contact the CML to ensure that appropriate diagnostic methods are employed. If a microbial biofilm is detected by

microscopy it may be reported using descriptive terms e.g. 'Microscopy shows Gram-negative rods in biofilm-like structures' (Figs 2, 4–10) and the species should be reported if specific fluorescence *in situ* hybridization probes have been used (Fig. 2) (AIII). In other cases where microbial biofilm infection is suspected (e.g. culture positive or PCR positive only following sonication or after scraping, rolling etc.) the findings may be reported as 'Growth of/PCR-detected microorganisms possibly from a biofilm infection'. Additionally the CML may offer assistance to the clinician regarding treatment regimens for biofilm infections e.g. 'Please contact CML or infectious diseases specialist for consultancy concerning treatment of the biofilm infection'.

Q1-5 Should routine antibiotic susceptibility testing employing planktonic bacteria from a biofilm infection be reported to the clinician? If yes—should the CML add an explanation about the interpretation of the results? How should such an explanation be written?

Routine antibiotic susceptibility testing—from disc diffusion to microdilution-based automatic methods—are performed with planktonically growing bacteria or *Candida* spp. and the breakpoints, e.g. Susceptible, Intermediate and Resistant (S-I-R) based on pharmacokinetics of the various antibiotics and the typical minimum inhibitory concentrations (MIC) of the relevant microorganisms are given (EUCAST). Biofilm-growing microorganisms are significantly more tolerant to antibiotics [6] and corresponding breakpoints have not been established [34]. The S-I-R results can therefore not be used to predict therapeutic success in the case of biofilm infections and offer no guide to clinicians for treating such infections. However, biofilm infections are often foci for systemically spreading infections, e.g. bloodstream infections originating from biofilms in intravenous lines or in urinary catheters or stents [110,111] (Fig. 5). Such systemic spread of biofilm infections can be treated successfully with antibiotics based on the results of routine antibiotic susceptibility testing, which should therefore be reported [112] (AIII). In that case, routine antibiotic susceptibility testing can efficiently predict treatment success or failure of the systemic infection (All). Clinicians should be informed by the CML that recurrence of the infection from the biofilm focus may occur if the focus cannot be removed or suppressed by antimicrobial treatment. This may be reported as, for example, 'Please contact CML or infectious diseases specialist for consultancy concerning treatment' (AIII).

Q1-6 Are there any routine *in vitro* antibiotic susceptibility tests that can reliably predict therapeutic success?

Biofilm susceptibility tests have been designed and include the Calgary device, where biofilms are grown on pegs protruding from the lid of a microtitre plate which are subsequently

exposed to various concentrations of antibiotics to establish their 'biofilm eradication concentrations'. However, such testing has not yet resulted in reliable prediction of therapeutic success [34,113–115] (DII).

Q1-7 Which research is urgently needed to improve diagnosis of biofilm infections?

- Algorithms for biofilm diagnosis and for handling the samples in the CML—why, when and how to demonstrate microbial biofilms.
- Improved non-invasive methods (e.g. image-based diagnostic methods) for detecting biofilms *in vivo* in patients.
- Host-specific markers that indicate a risk of biofilm infections.
- How many samples should be taken and for how long a period.
- How should samples be analysed for the detection of biofilm-growing microorganisms that may be viable-but-non-culturable in routine media.
- Which bacterial species play an active role in biofilm infections since culture-independent molecular methods may identify a greater microbial diversity than previously demonstrated by culture. To what extent do detected species come from DNA released from non-viable bacteria?
- Will enzymatic (e.g. DNases or other compounds) pretreatment of microbial biofilms improve diagnostic and therapeutic success?
- Characterization of biofilm-specific antigens and detection of specific antibodies against biofilm-specific antigens.
- Standardized biofilm susceptibility testing and establishment of biofilm-specific breakpoints for systemic and topically administered antibiotics.
- Do some mycobacteria cause biofilm infection in patients?
- Better animal models for relevant chronic biofilm infections [116].
- Assess the clinical impact of 16S rRNA gene detection in intravenous catheters [73,117,118]
- Is there any evidence that 'good bacteria' reduce the occurrence of biofilm infection (prevention of biofilm infection by e.g. probiotics)?
- Development of methods for the diagnosis of CR-BSI relying on the antigen-based detection of biofilm formation inside the catheter.
- Do surveillance cultures and light-microscopy studies of accumulated mucus within the ETT have any benefits on early detection of ETT biofilm formation and incidence of VAP and therapeutic outcomes?

Prevention and Treatment of Biofilm Infections

Generally

Antibiotics are used for:

- 1) *Prophylaxis* i.e. to prevent infection in patients who are not yet infected or colonized in, for example, the lungs or bones/joints, if there is an unacceptable risk of development of an infection in such organs during a specific period or during a specific (e.g. surgical) procedure; that means that the antibiotic penetrates the site of a potential infection before the microorganisms (e.g. surgical wound).
- 2) *Pre-emptive treatment* when colonization with a specific microorganism is detected on, for example, mucosal membranes and there is a known, unacceptable risk of development of severe clinical infection with that microorganism; that means that the antibiotic penetrates the site of a potential infection after the microorganisms, but before the establishment of an infection (e.g. pre-emptive therapy after open bone fracture to prevent biofilm infection on osteosynthetic material or pre-emptive eradication therapy of intermittent *P. aeruginosa* colonization in the lungs of CF patients to prevent biofilm infection).
- 3) *Empiric treatment* is based on the clinical diagnosis of infection without knowledge of the microorganism but covering the most probable microorganisms in a specific clinical situation (e.g. staphylococci and *E. coli* in case of CR-BSI).
- 4) *Definitive treatment* is based on clinical diagnosis and known culture and susceptibility testing results.

These definitions are used in the following section.

Q2-1 Can systemic antibiotics (oral, intravenous) be used to prevent some (which?) biofilm infections? Can the risk period be defined?

Yes for some infections, not for other infections.

Short-term (surgery prophylaxis, some urinary catheters) and intermediate-term (endotracheal tubes, some CVCs, some urinary catheters)

Patients with infections related to their orthopaedic alloplastic devices. Surgery-related biofilm infections can be prevented (their incidence reduced) by administration of prophylactic perioperative antibiotics (<http://www.sign.ac.uk/pdf/sign104.pdf>) [119] (A1).

Patients with indwelling urinary catheters or urethral stents. Short-course systemic antibiotic therapy can postpone biofilm infections for up to 1–2 weeks [120,121], however we do not recommend the use of antibiotic prophylaxis because of concern about superinfection by multiresistant strains [17,18] (DIII).

No systemic antibiotics can be recommended for the prophylaxis of CR-BSI [122–124] (DI).

There is no evidence to support the use of systemic antimicrobial agents to prevent biofilm infections in the treatment of wound-associated infections [50,125,126] (CIII).

There is no regimen that can be recommended regarding tissue fillers at the present time [29].

Patients with endotracheal tube biofilm VAP: Please see Q2-2.

Patients with intravenous catheters: see Antimicrobial lock therapy.

Chronic disposing situation (CF, chronic wound, late alloplastic infections, some CVCs (>1 year for, for example, parenteral feeding), some urinary catheters, implants/fillers (absorbable/material repairing defects temporarily). Chronic *P. aeruginosa* lung infection in CF cannot be prevented by prophylactic use of systemic antibiotics in patients before the onset of intermittent *P. aeruginosa* colonization [33] (DI). Urinary tract infections in patients chronically carrying urinary catheters cannot be prevented by prophylactic use of systemic antibiotic. No information currently exists about the other conditions.

Q2-2 Can topical use of antimicrobials or antimicrobials attached to the surface of foreign bodies (e.g. intravenous lines, urinary catheters, tracheal tubes, artificial joints, bone cements for orthopaedic surgery) be used to prevent some (which?) biofilm infections? Can the risk period be defined?

Short term (surgery prophylaxis, some urinary catheters).

Orthopaedic alloplastics—There is good evidence to suggest that antibiotic-impregnated materials (frequently gentamicin but also tobramycin and vancomycin) reduce the incidence of prosthesis-associated biofilm infections [24,127,128] (AI).

Short-term urinary catheters—Coating with antimicrobials (e.g. nitrofurals) can only postpone but not prevent biofilm infections [29] (AI). Several studies conclude that the postponing effect is sufficient to prevent biofilm infections in short-term catheterization especially with nitrofurazone [120,131] (AI).

Intermediate or long-term (endotracheal tubes, some CVC, some urinary catheters).

Endotracheal tube biofilm (VAP)—A few studies [12,132] have assessed the prophylactic effects of selective digestive decontamination, which involves the oropharyngeal/gastric administration of topical non-absorbable antibiotics, on ETT biofilm formation. There is consistent evidence that selective digestive

decontamination does not inhibit ETT biofilm formation, and we do not suggest its use as a prophylactic strategy (DI). Nebulized antibiotics achieve higher concentrations in the artificially ventilated airways (mean gentamicin concentration 1 h after nebulization of 80 mg was 790 µg/ml) than antibiotics administered by the parenteral route and in one clinical study [133], the use of nebulized gentamicin (80 mg in 4 ml saline every 8 h) in the prevention of VAP has been tested and it was effective in preventing the formation of ETT biofilm by the most common causative pathogens of VAP (CII). There have also been attempts to reduce ETT biofilm formation by coating the ETT surface with antimicrobial agents [63,134–136]. Among all the available antimicrobial compounds used for ETT coating, silver (dispersion of silver-sulphadiazine or micro-dispersed silver ions in a polymer) has been the main focus in laboratory and clinical investigations [44,134]. It has been shown that silver-coated ETTs could exert antimicrobial effects within the proximal airways and hinder ETT biofilm formation. In addition, the silver-coated ETT exerted its maximal effect during the first 10 days of tracheal intubation [44,136] and reduced mortality in patients with VAP [137,138] and the cost of VAP [61]. We recommend the use of silver-coated ETT in patients, who are expected to be ventilated for longer periods of time (≥1 week) and with risks for VAP (BI). The Mucus Shaver (National Institutes of Health, Bethesda, MD, USA) [61] has been devised to keep the ETT lumen free of mucus and to mechanically remove biofilm. It comprises an inflatable balloon with two or more 1.0-mm wide, 0.5-mm thick silicone rubber ‘shaving rings’. The balloon is inflated with air inside the ETT lumen sufficiently to force the two shaving rings firmly against the wall of the ETT. Thereafter the Mucus Shaver is gently retrieved followed by resumption of ventilation. A clinical study [43] in a small population of patients showed that the Mucus Shaver is efficient in ETT biofilm removal. In a recent study [46] in paediatric intubated patients, ETT biofilm was mechanically removed through the inflatable balloon of a urethral catheter. This strategy reduced ETT biofilm formation and incidence of clinically confirmed VAP. Based on the limited number of studies, we support the routine use of dedicated devices to mechanically remove ETT biofilm (BII).

Patients with CVCs—Use of chlorhexidine-impregnated sponges or dressings reduces the incidence of CR-BSI and is considered cost-effective [139–142] (AI). Coated CVCs should be considered if the incidence of CR-BSI is still high after implementation of all preventive measures. In that case, minocycline/rifampin-coated catheters are more efficient than chlorhexidine/silver sulphadiazine-coated catheters [143–146] (AI).

Preventive antibiotic lock technique—Antibiotic lock technique (ALT) is done by application of a small volume of concentrated antimicrobials, which is allowed to dwell for 12–24 h inside the catheter lumen. The use of ALT as prophylaxis should be restricted to patients who have experienced multiple CR-BSI

despite optimal aseptic techniques (BI). Minocycline-EDTA ALT is more efficient in reducing the rate of CR-BSIs in haemodialysis patients than heparin locks [147,148]. A mix of taurolidine/citrate/heparin gave a reduced rate of CR-BSI in haemodialysis patients compared with heparin [149,150]. Use of taurolidine/citrate without heparin is associated with an increased rate of thrombosis of the catheter and thereby significantly shorter catheter patency in patients undergoing haemodialysis [151]. In paediatric cancer patients, an early paper showed no significant reduction of total number of CR-BSI but a significant reduction of CR-BSI due to coagulase negative staphylococci with taurolidine/citrate compared with heparin [152], whereas a more recent paper showed a significant reduction of CR-BSI with taurolidine/citrate as compared with heparin if used from the time of insertion [153] (BI). In high-risk patients receiving parenteral nutrition, taurolidine/citrate reduces the rate of CR-BSI when initiated after the first episode of CR-BSI compared with heparin [154] (BI).

Use of ethanol as ALT—No significant reduction for ethanol 70% compared with heparin in reduction of CR-BSI incidence in haemodialysis was observed [155]. Among immunosuppressed haematology patients receiving chemotherapy, 70% ethanol significantly reduced CR-BSI compared with heparin in a randomized study [156]. In cases of high-risk patients using in-home parenteral nutrition (one previous CR-BSI), ethanol 70% was assessed in a before–after study with a significant reduction of CR-BSI and need for catheter replacement in children [157]. However, in a randomized placebo-controlled trial in adult haematology patients, no significant decrease in the incidence of CR-BSI and more adverse effects were reported in the ethanol group [158]. Furthermore, it has been reported that ethanol could increase preformed *S. aureus* biofilm *in vitro* [159]. A recent meta-analysis showed that ethanol was more effective than heparin lock to reduce the incidence of CR-BSI and catheter replacement in paediatric parenteral nutrition but may increase the risk of thrombosis [160]. In conclusion we recommend the use of ethanol lock (CII). *Antibiotic/antiseptic ointments*—The use of antibiotic ointments that have limited antifungal activity may serve to increase colonization and/or infection due to *Candida* species [161].

Regarding the prevention of fungal infections, the most promising ALT includes use of amphotericin B, ethanol or echinocandins [162]. (CII).

Indwelling urinary catheters—Coating with antimicrobials can postpone biofilm infections but not prevent them [17,79,130,163] (CI).

Chronic disposing situation (e.g. CF, chronic wound, late alloplastic infections, some CVC (>1 year for parenteral feeding, for example), some urinary catheters, implants/fillers (absorbable/material repairing defects temporarily)). Chronic *P. aeruginosa* lung infection in CF cannot be prevented by prophylactic use of nebulized

antibiotics in patients before the onset of intermittent *P. aeruginosa* colonization [33] (DI). Coating of long-term CVC or coating of indwelling urinary catheters and urethral stents by antibiotics or silver alloy cannot prevent chronic biofilm infection. The coating showed no or minimal effect on biofilm formation in several multicentre randomized controlled trials and cannot be recommended for common use [129,163] (DI).

There is little information regarding tissue implants/fillers where local irrigation has been used [29] (CIII).

Q2-3 Can some biofilm infections be prevented or early biofilm infections be eradicated by early antibiotic treatment of colonization (no symptoms or signs) with bacteria known to be able to cause biofilm infections in the type of patients in question?

Chronic P. aeruginosa lung infection in CF. The chronic infections can be prevented by pre-emptive systemic and/or nebulized antibiotic eradication therapy of intermittent *P. aeruginosa* colonization of the lungs [31,34] (AI).

Patients with intravenous catheters. Yes, ALT in case of repeated positive blood culture with identical coagulase-negative staphylococci or other microbes from CVC but no clinical signs of infection, also called CVC colonization [16,161] (CIII). There are no data that point to preference of specific antimicrobial agents.

Patients with VAP. No data available.

Patients with chronic wound infection. No data available.

Patients with infections related to their orthopaedic alloplastic devices. No data available.

Patients with indwelling urinary catheters and urethral stents. No data available.

Patients with infections connected to tissue fillers, e.g. breast implants. No data available.

Q2-4 Can some non-foreign-body-related biofilm infections be eradicated with antibiotics? Can some foreign-body (e.g. artificial joints) related biofilm infections be eradicated with antibiotics without removal of the foreign body? Can biofilm infections in critically important organs (e.g. lungs) or implants (e.g. aortic grafts) be eradicated by antibiotics? Or rather persistently suppressed by antibiotics? Are there any methods to evaluate if the treatment is successful and can be stopped?

Generally such biofilm infections afflict patients who need from a few weeks to life-long suppressive antibiotic treatment, if

eradication cannot be achieved. There is a risk of development of resistance due to conventional mechanisms (the risk is reduced by combination therapy at least initially when the number of microorganisms—and therefore risk of mutation—is higher) and of adverse events caused by antibiotic treatment. It is unknown which antibiotic concentration/dose is needed to suppress biofilm infection, whereas this is known in some diseases regarding treatment of spreading planktonic microbial infections originating from the biofilm focus and thereby achieving containment of the biofilm infection (e.g. CF with chronic *P. aeruginosa* lung infection which is a focal infection in localized areas of the lungs). Currently only clinical signs and symptoms and paraclinical tests (detection of the microorganisms by culture or culture-independent methods, imaging techniques, inflammatory parameters) are available for evaluation of the effect of antibiotic therapy. However, even if the surrogate parameters indicate a favourable response, the microorganisms in the biofilm may survive and give rise to relapse in the case of cessation of antibiotic therapy. Antibody levels may stabilize or decrease slowly in the case of successful therapy, but are not a reliable marker of successful treatment. Generally, there are no data concerning when or if the chronic suppressive antibiotic therapy can be stopped if the underlying condition is still present.

CF with chronic lung infections. Chronic *P. aeruginosa* lung infection can be eradicated in a few patients within the first 1–2 years after the onset of the infection [31,34] (All). This is not possible in most cases of chronic lung infection. In these patients, chronic suppressive antibiotic therapy is given as daily nebulized antibiotics combined with systemic antibiotics either regularly every 3 months or at acute exacerbations and the patients' lung function can thereby be maintained for decades [30,33,34] (AI).

Patients with infections related to their orthopaedic alloplastic devices (e.g. joint prosthesis). Some foreign-body related biofilm infections can be eradicated with antibiotics without removal of the foreign body, if duration of symptoms of infection is ≤ 3 weeks, implant is stable, there is an absence of sinus tract and microorganisms are susceptible to suitable biofilm-validated antibiotics followed by debridement and antibiotic combination therapy, which includes an agent with efficacy on biofilms. Rifampicins have this property against staphylococci and fluoroquinolones have this property against Gram-negative bacilli, but should be given as combination therapy with another antibiotic due to the risk of development of resistance [164]. Antibiotic combination therapy has to be combined with initial debridement surgery and exchange of modular parts of the implant [37] and has proved to be more effective than monotherapy [165,25,128,166]. This strategy is recommended (All).

Patients with intravenous catheters. Antimicrobial lock therapy is recommended in case of uncomplicated CR-BSI caused by coagulase-negative staphylococci, *Enterobacteriaceae* and possibly *P. aeruginosa* [167–171] (All). CR-BSI caused by *S. aureus* should lead to catheter removal because of a high risk of haematogenous complications, endocarditis and a low rate of treatment success in case of ALT [169,171,172,173]. CR-BSI caused by *Candida* spp. should also lead to catheter removal, because non-removal is associated with an increased mortality and a more prolonged candidaemia [16,102,162,175–182]. Even if catheter removal is recommended, many patients are not candidates for catheter replacement because of their general condition. The role of antifungal lock therapy against *Candida* is not well defined. Azoles have poor activity against *Candida* biofilms both *in vitro* and *in vivo*; lipid formulations of amphotericin B are more effective than amphotericin B deoxycholate, and echinocandins have excellent activity [161,182,183]. If conservative treatment with ALT is chosen, clinical status and blood cultures should be monitored to detect treatment failure, and therefore leading to catheter removal. In cases of CR-BSI, systemic antibiotics should always be associated with ALT (All).

Patients with endotracheal tube biofilm and VAP. Systemic antibiotic therapy is not effective in clearing the biofilm present on the ETT [26] (DII). In patients with VAP, the pulmonary infection is treatable when appropriate antibiotics guided by microbiological findings are promptly administered [26] (AI). The duration of therapy should be based upon the clinical response. In the majority of cases, a 7-day treatment period is appropriate. Nevertheless, in VAP caused by *P. aeruginosa* or methicillin-resistant *S. aureus*, a longer treatment up to 14–21 days is preferable. It is recommended to reassess the clinical response to therapy after 48–72 h and therapy should be promptly changed in the case of lack of clinical improvement [26] (AI).

Patients with indwelling urinary tract catheters or urethral stents. Antibiotic therapy is not sufficient to clear the biofilm present on the stent or catheter, but only decreases the number of microorganisms in the urine and results in suppression of the symptoms [174]. If the catheter is not removed or replaced, relapse will probably occur after the end of treatment, therefore antibiotic treatment without removal of stent or catheter cannot be recommended (All).

Patients with chronic wound infection. There is no reported evidence to support the use of systemic antimicrobial agents in the treatment of chronic wound-associated infections and their healing, even if this therapeutic approach is quite common in clinical practice [50,125,126] (CIII).

Patients with infections connected to tissue fillers e.g. breast implants.
No data available.

Q2-5 What is the optimal antibiotic strategy for treatment of established biofilm infections?

Cystic fibrosis. Chronic suppressive therapy with nebulized antibiotics and systemic antibiotics either regularly every 3 months, or at acute exacerbations. Systemically administered antibiotics yield concentrations in the respiratory compartment of the lungs, which are adequate for planktonic bacteria, although insufficient for biofilm-growing bacteria, whereas the concentrations reached in the conductive compartment of the lungs (bronchi, sputum) are low. Topically administered antibiotics by inhalation, on the other hand, provide very high concentrations in the conductive compartment of the lungs, but low concentrations in the respiratory compartment. Combinations of systemic and topical antibiotics are therefore frequently used to reach both the respiratory and the conductive compartments of the lungs [30,31,33,34,184] (AI). Pharmacokinetics/pharmacodynamics of antibiotics against biofilm-growing microorganisms has only been carried out in animal studies and only with a few antibiotics, and the results indicate that β -lactam antibiotics show time-dependent killing and ciprofloxacin, colistin and tobramycin show concentration- or dose-dependent killing of biofilm-growing *P. aeruginosa* cells similar to planktonic growing cells. However, the concentrations of antibiotic needed were, in all cases, much higher even in the case of antibiotics with time-dependent killing [185–187].

Patients with chronic wound infection. There is a lack of systematic studies. There is a need to clarify when to treat (obvious signs of infection versus low grade with few signs of infection) and what to treat (e.g. which bacteria or fungi are pathogenic?). All patients need non-antimicrobial strategies (e.g. debridement, compression, vacuum therapy etc.). If treatment is considered necessary, combination therapy may be more effective (two antibiotics with different mechanisms of action, systemic + local treatment, antibiotic + local disinfectant). While the rationale for debridement seems logical, the evidence to support its use to enhance healing is scarce. There is more evidence in the literature on the effectiveness of debridement for diabetic foot ulcers than for venous ulcers and pressure ulcers. Additional studies are needed to provide clinical evidence for debridement inclusion in surgical treatment protocols for chronic wounds [188] (BIII). After debridement, topical antimicrobial agents may be more effective in the treatment of the infected wound and in avoiding the re-establishing of microbial biofilm [189] (BI). Negative pressure wound therapy with irrigation or instillation may lower the bacterial burden in chronic wounds and prevent biofilm formation (CIII).

Patients with infections related to their orthopaedic alloplastic devices. There is probably a time-dependent success rate in the case of implant retention. Based on observational studies, critical timing is set at 3 weeks after start of symptoms (haematogenous infections) or 4 weeks after implantation (perioperative infection) [37]. Debridement, implant retention and long-term antimicrobial therapy in patients with acute infections have a success rate of at least 85%. For these high cure rates, treatment with active antibiotics (rifampicin against staphylococci, fluoroquinolones against Gram-negative bacilli) is required [38]. In chronic infections (>3-week duration or >4 weeks after surgery), prosthetic devices should be replaced [37] after thorough debridement. In the case of two-stage exchange, local therapy with an antibiotic-containing cement spacer is generally used. In the case of one-stage exchange or two-stage exchange with a short interval (2–3 weeks), long-term antimicrobial therapy with suitable biofilm-validated antibiotics should be used [190]. Duration of treatment is not well established but a treatment period of 6–12 weeks is well accepted (BIII). If the implant is not replaced before 2 months, 6 weeks of antimicrobial therapy is enough. No biofilm-active regimen is required after careful removal of all foreign material (BI).

Patients with endotracheal tube biofilm VAP. A detailed description of adequate therapy in patients with VAP is beyond the scope of these guidelines, and it is reported elsewhere [26]. Appropriate and prompt antibiotic therapy significantly improves survival for patients with VAP. Importantly, antibiotics with good pulmonary penetration should always be considered as first-line treatment. Antimicrobial selection should be based upon risk factors for multidrug-resistant (MDR) pathogens. In patients at risk for MDR pathogens, empiric broad-spectrum, multidrug therapy is recommended. Therapy can be de-escalated as soon as the causative pathogen is identified and antibiotic susceptibility can be assessed. Combination therapy against Gram-negative pathogens could provide a greater spectrum of activity and should be indicated based on the patient risks for MDR pathogens and local prevalence of MDR pathogens. Additionally, in institutions where methicillin-resistant *S. aureus* is frequent, appropriate empiric antibiotic therapy should be considered (AIII).

Patients with intravenous catheters. In the case of ALT performed for biofilm eradication, the length of treatment is between 7 and 14 days [169–171,191] (AII). There are no data that point to preference of specific antimicrobial agents. Systemic antibiotics should always be associated with ALT in case of conservative treatment (AII). Antibiotic concentration used for ALT should be 100 to ≥ 1000 -fold MIC [192]. Time of contact

between ALT and catheter should be between 12 and 24 h. No data favours the adjunction of heparin to antibiotic. An *in vivo* study demonstrated that heparin did not increase the effect of vancomycin or ciprofloxacin effect in ALT [193].

Patients with indwelling urinary catheters or urethral stents. Use of renally excreted antibiotics together with change of the catheter or stent (AI). The recommended duration of the antibiotic treatment is 7 days for patients with catheter-related urinary tract infections who have prompt resolution of symptoms and 10–14 days for those with delayed response (AIII) [18].

Patients with infections connected to tissue fillers e.g. breast implants. No information available.

Q2-6 How can the treatment effect be monitored?

See Q2-1 to Q2-3. By clinical signs and symptoms and para-clinical tests such as detection of microorganisms from samples obtained from the focus of biofilm infection by culture or culture-independent methods, imaging techniques, inflammatory parameters, improvement of organ function available for evaluation of the effect of antibiotic therapy (e.g. lung function measured by FEV1) [30,31,33,34]. However, even if the surrogate parameters respond favourably, microorganisms in the biofilm may survive and give rise to relapse after cessation of antibiotic therapy according to the experience from CF patients with chronic *P. aeruginosa* biofilm lung infection [30,31,33,34] (AI). Antibodies may stabilize or decrease slowly in cases of successful therapy, but this is not a reliable marker in that respect. Generally, except in CF, where antibiotic therapy (named chronic suppressive therapy or maintenance therapy) is life-long in the case of chronic biofilm infection [30,31,33,34] (AI), there are no data concerning when or if the chronic suppressive antibiotic therapy can be stopped if the underlying condition is still present.

Q2-7 Which research is urgently needed to improve prevention and treatment of biofilm infections?

- New anti-biofilm effective antibiotics and determination of pharmacokinetics/pharmacodynamics for existing and new anti-biofilm antibiotics on young and old biofilms including combination antibiotic therapy.
- New anti-virulence drugs (for example drugs with quorum sensing inhibiting properties) and determination of their pharmacokinetics/pharmacodynamics on new and established biofilms including a combination of antibiotics and anti-virulence drugs.
- Anti-inflammatory approaches (or modification of established approaches) for biofilm infection therapy, since much of the tissue damage around biofilm infections is due to the host inflammatory response.
- Biofilm matrix degrading or dissolving drugs such as enzymes or chelators of components of biofilms with the purpose of rendering the biofilm to a planktonic state, amenable to antibiotic treatment.
- New combinations of antibiotics combined with biofilm-dissolving drugs.
- Topical antimicrobial treatment regimens.
- Combination of antimicrobials with ultrasound, electricity or UV light.
- Leucopatch (local application of autologous polymorphonuclear leucocytes and platelet-rich fibrin) on biofilm infections in diseases with impaired circulation e.g. infected chronic ulcers [194].
- Maggots (for selective debridement) in combination with antimicrobials or quorum sensing inhibitors to treat biofilm infections in, for example, chronic ulcers.
- Vaccination with relevant biofilm-forming bacteria to prevent establishment of such infections inspired by the moderate success in trials of *P. aeruginosa* vaccines against chronic biofilm infections in CF patients [195].
- Investigation of the period needed *in vitro* and *in vivo* to treat biofilms to achieve eradication (if possible) or permanent suppression with the purpose of establishment of standardized biofilm treatment.
- Investigate the effects of dedicated devices to mechanically remove ETT biofilm, i.e. the Mucus Shaver, on the prevention of VAP.
- Investigate therapeutic benefits of nebulized antibiotics on ETT biofilm and VAP.
- Investigate how to monitor success of antibiotic treatment of biofilm infections.
- Establishment of animal models that realistically reflect chronic biofilm infections in humans.
- Investigate whether clinically relevant antimicrobial surfaces can be developed for the long-term prevention of biofilm infections on medical foreign bodies (e.g. intravenous lines, catheters, tracheal tubes, alloplastic materials).
- Since biofilms are microaerophilic or anaerobic below their surface and since the bactericidal activity of many antibiotics involves reactive oxygen/hydroxyl species, the influence of hyperbaric oxygen therapy on antibiotic treatment of biofilms should be studied.
- Assessment of new antibiofilm compounds against CR-BSI such as ethanol and chelators.
- Evaluation of ALT in the clinical setting since data focusing on the activity of antifungal agents and other biocides on fungal biofilms mainly correspond to *in vitro* or *in vivo* (animal models) experiments.

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