REVIEW ARTICLE

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Outcome of Antimicrobial Therapy in Documented Biofilm-Associated Infections

A Review of the Available Clinical Evidence

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Contents

Abstract

Numerous laboratory findings indicate that microbial biofilms may be encountered in several types of human infections, affecting the activity of antimicrobial agents. We evaluated the clinical evidence regarding the effectiveness of antimicrobial therapy for infections documented to be biofilm-associated, by performing a review of 15 relevant studies, excluding dental and eye infections. In a clinical trial, a significant difference was noted in the effectiveness of antibacterial agents used for catheter-related urinary tract infections in which substantial bacterial adherence on uroepithelial cells was observed. In case series and case reports, 28 patients with biofilm-associated infections documented by electron microscopy scanning were identified. Infection sites included ear, urinary tract, CNS, bloodstream and foreign body implantation site. *Pseudomonas* and *Staphylococcus* spp. were the predominant microorganisms among the bacterial or fungal causative pathogens. In 24 cases, infections related to the presence of foreign bodies. Treatment failure or recurrence was noted in all eight patients in whom targeted antimicrobial therapy was instituted before foreign body removal. Foreign body removal coupled with antimicrobial therapy was effective in all ten relevant

cases. In four cases of native tissue urinary tract infections, the outcome of the initial antimicrobial therapy was poor. The limited available relevant clinical evidence indicates that conventional antimicrobial therapy alone is not adequately effective against documented biofilm-associated infections. Although some regimens might be more appropriate in this setting, further research on novel therapeutic strategies is needed to improve the outcome of patients with biofilm-associated infections.

Over the years, numerous studies have focused on the elucidation of the composition and structure of microbial biofilms, as well as the complex interactions within the various constituents of the biofilms and between biofilms and their surrounding environment. It is well recognized that microbial biofilms constitute a structured community of microbial cells encased in a self-produced extracellular polymeric substance and adherent to an abiotic or biotic surface.[1] Biofilms may be composed of single or multiple microbial species.[1] Within biofilms, which are commonly heterogeneous in structure, microbial cells mainly form matrix-enclosed clusters between intercalated water channels.[2] The latter facilitate nutrient and oxygen transport from the surrounding environment to the cell clusters.[3]

Bacteria or fungi are found in a sessile state within biofilms and have an altered profile of gene expression compared to planktonic (free-living) counterparts.[4,5] Cells in different regions of a biofilm may have specialized biological activity.[1] The heterogeneous function of cells constituting a biofilm is primarily coordinated by quorum-sensing mechanisms.[6] Small molecules secreted by microbial cells with autocrine- and paracrine-like activity, called autoinducers, are considered to mediate cell-to-cell communication within biofilms.[7]

Major attention has been given to the role of microbial biofilms in various types of infections affecting humans. A fundamental role of microbial biofilms has been asserted in foreign body infections in particular. This has been mostly based on the demonstration of the presence of microbial biofilms on the surface of various types of medical devices removed from sites of infection.[2] Furthermore, the presence of microbial biofilms has been recognized in several types of native tissue infections, including bronchopulmonary infections in patients with cystic fibrosis, as well as middle-ear infections, chronic rhinosinusitis, diabetic foot infections, urinary tract infections, bacterial vaginosis, endocarditis and periodontitis.[2,8-14] In total, it has been postulated that microbial biofilms may be present in more than 60% of microbial infections.[15] Several species of bacteria and fungi have been identified as causative pathogens of biofilm-associated infections in humans. The prevalence of these pathogens differs by the type of infection encountered.[1] The clinical relevance of biofilm-associated infections is further indicated by the fact that causative pathogens typically show reduced susceptibility *in vitro* to antimicrobial agents and host defense mechanisms.[2]

It should be mentioned that the current understanding of the clinical relevance of biofilm-associated infections is mainly based on indirect evidence derived from laboratory findings, as well as on clinical observations regarding foreign body infections, which are presumed to be biofilm-associated. In this context, we reviewed the available published clinical evidence regarding the outcome of antimicrobial therapy in patients with infections that were documented by appropriate methods to be related to the presence of microbial biofilms.

1. Literature Search

We searched Medline using PubMed through to August 2008 for clinical case reports, case series, non-comparative and comparative studies as well as clinical trials that evaluated the clinical outcome of patients with infections associated

with biofilm formation. The main search involved the use of the following search terms: 'biofilms' AND ('antimicrobial' OR 'antibiotic'). Additional searches involved the combination of the term 'biofilms' with terms referring to the names of different antimicrobials, as well as a search on 'biofilms' with a limit for article types consisting of clinical trials or case reports.

Only studies that evaluated the clinical outcome of patients who received treatment for bacterial or fungal infections that had to be aetiologically related to the formation of biofilms, as documented by microscopic methods, were eligible for evaluation in this review. Studies referring to dental or eye infections were excluded. Studies reported as conference abstracts or published in languages other than English were also excluded.

We initially screened, based on title and abstract, 1465 articles that were retrieved by applying the relevant searches performed in PubMed. Finally, 15 articles were identified as eligible for inclusion in this review.[16-30] These consisted of one clinical trial,[16] two retrospective case series[20,27] and 12 case reports.[17-19,21-26,28,30]

2. Results

2.1 Clinical Trial

In a double-blind trial, 42 hospitalized, paraplegic or quadriplegic, spinal cord injury patients with catheter-associated symptomatic urinary tract infections were randomized to receive treatment for 7 days with either ofloxacin or cotrimoxazole (trimethoprim/sulfamethoxazole).[16] Alternative antibacterials were administered in the cotrimoxazole group when the causative pathogens were shown to be resistant to this agent. The majority of the causative pathogens were Enterobacteriaceae (66.7%), while the remainder consisted equally of non-fermenting Gram-negative bacteria and Gram-positive cocci. Thirty-nine patients had laboratory findings indicative of biofilm formation. The latter was assumed by the demonstration of at least 10–20 adherent bacteria per uroepithelial cell with light microscopy. At day 7, ofloxacin treatment resulted in superior clinical success rate (90% vs 57%; $p = 0.015$) compared with cotrimoxazole or alternative agents (which, of note, were given to four patients).

2.2 Case Series and Case Reports

2.2.1 Infection Types

In table I, we present the characteristics and treatment outcomes of the 28 patients with documented biofilm-associated infections who were identified in relevant case series studies or case reports.[17-30] In all of these patients, the formation of a biofilm was documented by means of electron microscope scanning. Twelve of these patients were reported in a single retrospective case-series study representing children with post-tympanostomy tube placement-associated otorrhoea.[20] Four other patients also had ear infections,[18,21-23] while five patients had urinary tract infections,[24,26,27] two patients had CNS infections,[19,25] two patients had a penile prosthesis infection,[29] and the remaining three patients had a secondary bloodstream infection,[30] a prosthetic joint infection[17] or a mandibular implant infection.[28] In 24 of the total 28 identified cases, the index infection was related to the presence of a foreign body,[16-23,25,26,28-30] and biofilm formation was demonstrated on the surface of the associated foreign body. The four exceptions with native tissue infections involved three patients with chronic bacterial prostatitis unresponsive to targeted antimicrobial therapy who were reported in a case series study[27] and one patient with recurrent episodes of pyelonephritis.[24] Biofilm formation was demonstrated on the ductal and acinar walls of prostate tissue in the former three cases, and on teflon material placed in contact with urine for 3 days in the latter case. Regarding the latter case, it should be noted that multiple invasive procedures had been performed at the infection site prior to the development of the biofilm-associated infection.[24]

2.2.2 Causative Pathogens

The microorganisms identified as causative pathogens for the biofilm-associated infections outlined in section 2.2.1 included *Pseudomonas* spp. in all of the 12 patients in the case series study as well as in six additional cases,[18,20,23,24,26,27,29]

Table I. Characteristics of cases of documented biofilm infections and outcome of antimicrobial therapy

Study	Subjects (age, sex, no.)	Biofilm infection	Associated clinical characteristics	Foreign body	Biofilm documentation (material)	Causative pathogens (isolation site)	Initial potentially effective antimicrobial therapy	Outcome	Antimicrobial therapy after foreign body removal	Outcome
Stoodley et al.[17]	47 y, M	Prosthetic joint infection	Comminuted distal humeral fracture, bone non-union and failure of internal fixation and reduction	Total elbow arthroplasty with allograft	Electron microscopy (wound tissue and fluid, bone cement)	Meticillin-resistant *Staphylococcus aureus* (joint fluid)	None[a]	Recurrence after foreign body removal, irrigation, debridement and antibacterial impregnated bone cement spacer placement	Vancomycin plus rifampicin	Cure
Barakate et al.[18]	5 y, M	Persistent otorrhoea	NR	Tympanostomy tube	Electron microscopy (tube)	*Pseudomonas aeruginosa*	Various courses of topical and systemic antibacterials	Persistence	Type not specified	Cure
Bayston et al.[19]	~18 y, M	Ventriculitis	History of hydrocephalus	Ventriculo-peritoneal shunt	Electron microscopy (shunt)	*Propionibacterium acnes* (CSF, fluid aspirated from shunt)	Penicillin followed by long-term suppressive therapy	Recurrence after 7 y	Penicillin	Cure
Jang et al.[20]	12 pts,[b] M = 7	Persistent otorrhoea	NR	Tympanostomy tube	Electron microscopy (tympanostomy tube)	*P. aeruginosa* (fluid from otorrhoea)	None[a]	Failure	NR	NR
Pawlowski et al.[21]	5 y, F	Cochlear implant infection	Hearing loss, cochlear implant replacement	Cochlear implant device	Electron microscopy (implant surface)	*S. aureus* (wound)	Amoxicillin/clavulanic acid perioperative prophylactic therapy	Development of infection	NR	Cure
							Systemic ceftriaxone, topical ciprofloxacin	Failure		

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Table I. Contd

Study	Subjects (age, sex, no.)	Biofilm infection	Associated clinical characteristics	Foreign body	Biofilm documentation (material)	Causative pathogens (isolation site)	Initial potentially effective antimicrobial therapy	Outcome	Antimicrobial therapy after foreign body removal	Outcome
							Added systemic targeted therapy	Failure		
Cristobal et al.[22]	10 mo, F	Cochlear implant infection	Beckwith-Wiedemann syndrome, history of chronic suppurative otitis media	Cochlear implant device	Electron microscopy (implant surface and electrode array)	*Candida albicans* (middle-ear fluid)	None	NA	Liposomal ampho-tericin B	Sustained resolution, cochlear re-implantation ~6.5 after infection
Bothwell et al.[23]	4 y, NR	Persistent otorrhoea	Chronic intermittent ear drainage, adenoidectomy	Tympanostomy tube (replaced)	Electron microscopy (tube)	*Pseudomonas* spp. (otorrhoea fluid)	Systemic intravenous therapy	Failure	Topical quinolone	Cure after 1 wk
Furuhata et al.[24]	38 y, M	Recurrent pyelonephritis	Ureteropelvic junction stricture, ureteral stone, pyeloplasty, lithotomy, resection of ureteral segment, placement of temporary ureteral stent and nephrostomy catheter	None	Electron microscopy (teflon in contact with urine for 3 d)	*P. aeruginosa, S. epidermidis* (urine)	Piperacillin	Resolution, recurrence	NA	NA
							Cefpirome	Resolution, recurrence		
							Panipenem/ betamipron	Resolution, recurrence		
							Clarithromycin followed by clarithromycin combined with imipenem/ cilastatin	Cure		

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Table I. Contd

Study	Subjects (age, sex, no.)	Biofilm infection	Associated clinical characteristics	Foreign body	Biofilm documentation (material)	Causative pathogens (isolation site)	Initial potentially effective antimicrobial therapy	Outcome	Antimicrobial therapy after foreign body removal	Outcome
Davis et al.[25]	52 y, M	Meningitis (recurrence)	History of coccidioidal meningitis with obstructive hydrocephalus	Ventriculoperitoneal shunt	Electron microscopy (ventriculo-peritoneal shunt tubing)	*Coccidioides immitis* (CSF)	Fluconazole long-term maintenance therapy	Recurrence 5 y after initial episode	Fluconazole for months	Gradual improvement, sustained for at least 2 y
Stickler et al.[26]	63 y, F	Asymptomatic bacteriuria	Multiple sclerosis, diabetes mellitus	Urinary catheter	Electron microscopy (catheter casts)	*P. aeruginosa, Escherichia coli, Enterococcus faecalis, Proteus mirabilis*	None	Recurrence after changing catheters	Ciprofloxacin	Improvement
Nickel and Costerton[27]	3 pts, M	Chronic bacterial prostatitis	NR	None	Electron microscopy (perineal biopsies of prostate)	*E. coli*, *P. aeruginosa*, coagulase-negative *Staphylococcus* sp. (one pathogen in each case)	Various agents	Failure	NA	NA
Nishioka et al.[28]	69 y, F	Mandibular implant infection	Prosthetic rehabilitation	Mandibular subperiosteal implant	Electron microscopy (implant)	Not cultured	Cefazolin[c]	Partial resolution, recurrence	Cefazolin[c]	Cure
Nickel et al.[29]	32 y, M	Surgical site infection	Quadriplegic, bladder sphincterectomy, urinary tract infection	Penile prosthesis	Electron microscopy (prosthesis)	*P. aeruginosa* (prosthesis)	None[d]	Deterioration	NR	Cure
	58 y, M	Penile prosthesis infection, erosion into urinary bladder	Diabetes with retinopathy, nephropathy	Penile prosthesis	Electron microscopy (penile prosthesis)	*S. aureus* (urine)	Cephalexin	Failure	Cotrimoxazole	Cure
							Amoxicillin	Failure		

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Table I. Contd

Study	Subjects (age, sex, no.)	Biofilm infection	Associated clinical characteristics	Foreign body	Biofilm documentation (material)	Causative pathogens (isolation site)	Initial potentially effective antimicrobial therapy	Outcome	Antimicrobial therapy after foreign body removal	Outcome
Marrie et al.[30]	56y, M	Secondary bloodstream infection	Resolving soft tissue infection after elbow injury, history of syncopy attacks	Implanted transvenous pacemaker	Electron microscopy (distal portion of pacemaker lead)	*S. aureus* (blood)	Cloxacillin	Recurrence after 1 wk	Cloxacillin	Cure
							Cloxacillin, rifampicin	Recurrence 9 d after hospital discharge		

a Causative pathogens were resistant to agents administered.

b Children.

c Empirical therapy.

d We did not consider initial therapy as adequate for the pathogen finally isolated.

CSF = cerebrospinal fluid; **F** = female; **M** = male; **NA** = not applicable; **NR** = not reported.

along with *Staphylococcus* spp. in six more cases,[17,21,24,27,29,30] Enterobacteriaceae in two cases,[26,27] other Gram-positive bacteria in two cases[19,26] and fungal species in two cases.[22,25] No pathogen was isolated from routine culture specimens in one case.[28] More than one causative pathogen of the biofilm-associated infection was identified in two of these 28 cases.[24,26]

2.2.3 Treatment and Outcomes of Biofilm-Associated Infections

Among the 24 cases in which the biofilm-associated infection was related to the presence of a foreign body, eight received antimicrobial therapy potentially effective against the causative pathogens prior to the removal of the associated foreign body. However, antimicrobial therapy was ineffective in three patients with ear infections, as well as one patient with bacteraemia due to an infected implantable transvenous pacemaker lead, and in two other patients with infection at the site of foreign body implantation.[18,21,23,28-30] In two additional cases in which antimicrobial agents were administered as long-term maintenance therapy, recurrence of the original infection was noted.[19,25] It should also be noted that in the series of 12 cases with persistent otorrhoea due to *Pseudomonas aeruginosa,* topical ciprofloxacin failed to resolve the infection.[20] This could be attributed to the fact that the respective isolates in all these patients were resistant to ciprofloxacin.

Removal of the foreign body associated with the formation of a biofilm, coupled with targeted antimicrobial therapy against the causative pathogens, resulted in cure or improvement of the infection in all ten cases for which specific relevant data were reported.[17-19,22,23,25,26,28-30] However, in four of these cases the antimicrobial regimen administered after the removal of the foreign body had also been administered prior to the removal of the foreign body without clinical success.[19,25,28,30]

In the cases in which documented biofilm infections were not associated with the presence of foreign bodies, antimicrobial therapy failed in the three with chronic bacterial prostatitis,[27] whereas it was associated with multiple recurrences,

but eventually led to cure, in the patient with pyelonephritis.[24]

The type of antimicrobial therapy associated with cure or improvement of the documented biofilm-associated infections in the cases included in this review for which specific relevant data were available, was monotherapy in six cases[19,22,23,25,26,30] and combination therapy in the other two cases.[17,24]

3. Discussion

Despite the relatively extensive amount of literature evaluating the properties of biofilms *in vitro*, the data we identified regarding the clinical outcome of antimicrobial therapy in patients with appropriately documented biofilm-associated infections were limited. According to the relevant case series or case reports identified, biofilms are associated with various types of infections in humans. These infections are mainly related to the presence of foreign bodies, but may develop on native tissues as well. Various bacterial and fungal species may be the causative pathogens of biofilm-associated infections, while biofilms can be polymicrobial. As shown in the great majority of the identified cases, targeted antimicrobial therapy alone, either monotherapy or combination therapy, is ineffective in clearing the infection. Even in patients initially responding to antimicrobial therapy alone, recurrence may be observed. However, antimicrobial therapy accompanied by surgical removal of the foreign body associated with the biofilm infection, may be effective in the majority of patients. In addition, according to a clinical trial identified in our review,[16] there might be considerable difference in the clinical effectiveness of the various antimicrobial agents against biofilm-associated infections caused by susceptible pathogens.

Several factors are considered to compromise the effectiveness of antimicrobial agents against pathogens present in biofilms.[1,2] The fact that biofilms constitute a barrier to the action of host immune mechanisms may limit the effectiveness of bacteriostatic antibacterials.[31] Additionally, certain antibacterials, such as aminoglycosides, show delayed or reduced penetration into biofilms. While others may adequately diffuse into biofilms through water channels,[2,32,33] it is not well established whether they can adequately penetrate into microbial cell clusters.[3,34] The activity of certain antimicrobial agents may also be compromised under conditions of low oxygen tension or low pH, existing in local microenvironments of biofilms.[35,36]

The limited effectiveness of antimicrobial therapy in eradicating the causative pathogens of biofilm infections, which is reflected in the relatively high recurrence rate, may also relate to the presence within biofilms of microbial subpopulations with a substantially slow growth rate.[37,38] Such 'persister cells' exhibit downregulation of several biosynthetic processes that constitute targets of certain antimicrobial agents.[36] Furthermore, microbial subpopulations in biofilms commonly show phenotypic variations, attributed to a 'stress response' gene expression programme elicited under conditions of poor nutrient supply, increased cell density or osmotic stress.[2,34] This may result in increased expression of β-lactamases and efflux pumps, or reduced rate of cell wall synthesis.[31,39] Pathogens within biofilms may also have high mutation rate or easily exchange genetic material, thus facilitating the development of antimicrobial drug resistance.[31,40,41]

The lack of adequate effectiveness of conventional antimicrobial therapy against biofilm-associated infections has led to the evaluation of novel therapeutic strategies. Agents to which the planktonic forms of pathogens are resistant may inhibit some of the processes involved in the formation of biofilms. For example, clarithromycin has shown such activity *in vitro* as well as in combination with ofloxacin in an experimental rat model of infection, against *P. aeruginosa* biofilms.[42] This strategy was applied in practice in one of the cases included in our review with a favourable outcome.[24]

Another experimental therapeutic approach against biofilm-associated infections involves the application of physical agents, such as electric current or ultrasound, to enhance the activity of antimicrobial agents against biofilm bacteria by disrupting the extracellular matrix or facilitating drug penetration.[43,44] Thrombolytic agents have

also been used *in vitro,* mainly to disrupt the integrity of fibrin-rich biofilms.[45] Other proposed therapeutic strategies include the use of quorum-sensing inhibitors, such as *N*-acyl-homoserine lactone inhibitors or the RNA-III inhibiting peptide,[46,47] the cyclic administration of antimicrobials to mobilize and thus eradicate 'persister cells', the development of compounds with activity against 'persister' cells or the extracellular substance of biofilms,[15,31,32] or of monoclonal antibodies inhibiting microbial processes involved in biofilm formation.[48] Last but not least, various methods have been developed for the prevention of biofilm infections that are associated with the implantation of foreign bodies.[31]

The main limitation of this review evaluating the outcome of antimicrobial therapy in documented biofilm-associated infections is that the relevant identified clinical data were rather limited. This could be attributed to the difficulty in demonstrating the presence of biofilms in clinical samples. The main relevant method used in the studies included in our review was electron microscope scanning. Although electron microscopy provides detailed information for the study of biofilms,[49] it is not routinely available in microbiological laboratories for the diagnosis of biofilm-associated infections. Additionally, the demonstration of a substantial number of adherent bacteria on epithelial cells that was used in the clinical trial included in our review is a rather loose criterion for the diagnosis of biofilm-associated infections, since it is indicative only of the initial stage of biofilm formation.

Furthermore, the microscopic documentation of biofilm-associated infections[50] typically requires invasive procedures to be performed to obtain suitable material for examination. Thus, the documentation of biofilm formation may be more likely for cases of biofilm infections associated with the presence of a foreign body, compared with native tissue infections. In this regard, the former category of infections is expected to be overrepresented in the literature. Similarly, the potential presence of biofilms in patients with infections that are cured with antimicrobial therapy alone (without the additional need for surgical intervention) is expected to be underreported. The development and use of methods to identify the presence of microbial biofilms in routine microbiological specimens is expected to promote our knowledge regarding the clinical characteristics and outcomes of biofilm-associated infections. Nucleic acid amplification methods for the detection of specific quorum-sensing signals may particularly aid in this regard.[51]

4. Conclusions

In this review, we identified and evaluated the published evidence regarding the outcomes of infections documented to be associated with the presence of microbial biofilms. The findings derived from the synthesis of the rather scarce relevant data are in accordance with current concepts on infections presumed to be biofilm-associated. Specifically, most of the cases identified in our review involved infections associated with the presence of foreign bodies, rather than native tissue infections. Antimicrobial therapy targeting the causative pathogens, either bacteria or fungi, was generally ineffective, unless combined with the removal of the infected foreign bodies. On the basis of the data considered in this review, we could not identify specific antimicrobial agents with adequate effectiveness against biofilm-associated infections. Thus, additional clinical data regarding the characteristics and outcomes of infections related to the presence of microbial biofilms are required. The collection of such data could be facilitated by the development and use of appropriate methods to detect the presence of biofilms in routine clinical specimens. Further research on novel therapeutic strategies for biofilm infections is also needed.

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