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SHORT COMMUNICATION

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A microbiological and confocal microscopy study documenting a slime-producing *Staphylococcus epidermidis* isolated from a nylon corneal suture of a patient with antibiotic-resistant endophthalmitis

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Abstract *Background:* We describe a case of posttraumatic endophthalmitis unresponsive to systemic (amoxicillin+clavulanic acid and piperacillin/tazobactam), intra-ocular (vancomycin) and topical (ofloxacin, tetracycline and sulfametoxazole) antibiotic therapy. Microbiological and confocal microscopy studies of a nylon corneal suture revealed the presence of a slime-producing strain of *Staphylococcus epidermidis*. *Methods:* We describe the history and clinical presentation of a 77-year-old man in whom a high-grade posttraumatic endophthalmitis resolved only after the removal of a single nylon corneal suture. Microbiological investigations of the aqueous, vitreous and suture were performed, and the propensity of the suture-associated isolate to form biofilm was assessed using confocal microscopy. *Results:* A single stain of *S. epidermidis* was isolated from both aqueous and vitreous specimens and from the suture. The planktonic form of the isolate was susceptible in vitro to the antibiotics administered to the patient, but the strain was capable of forming biofilms and this phenotype showed resistance to high concentrations of the same antibiotics. *Conclusions:* The presence of a slime-producing strain of *S. epidermidis* should be considered in endophthalmitis that is unresponsive to specific antibiotic therapy, especially in cases in which an intra-ocular foreign body (e.g., a suture) is present.

Keywords Endophthalmitis · Biofilm · Slime · *Staphylococcus epidermidis* · Confocal microscopy

Introduction

Bacteria can grow both as floating cells in a liquid environment (planktonic growth) and as adherent microcolonies that can evolve to form large biofilms at solid–liquid interfaces (sessile growth) [10]. Biofilm formation requires the adhesion of bacteria to a solid structure, followed by the bacterial production of a polysaccharide glycocalyx (slime) that prevents antibiotics from gaining access to the microorganisms and reduces the efficacy of host defenses [4, 6, 10]. Biomedical devices are among the most widely studied solid surfaces that can be colonized by bacteria, with consequences that have been long underestimated, but can often be serious. In fact, sessile growth of bacteria can cause silent

chronic infections involving tissues surrounding a medical device which can exacerbate or cause acute life-threatening infections when planktonic cells are dispersed from the biofilm [4, 10].

Several studies have documented that bacteria can form biofilms on various intra-ocular lenses [7], leading to suggestions that this mode of growth may be involved in the etiology of postcataract surgery endophthalmitis or pseudophakic chronic intra-ocular inflammation. However, the presence of a slime-producing bacterium has never been documented on a device removed from an infected eye. In addition, biofilm formation has never been documented in other frequently used ophthalmic prostheses, such as sutures. Here we describe a case of endophthalmitis, unresponsive to specific antibacterial therapy, in which (for the first time) microbiological and confocal microscopy study of a nylon corneal suture revealed the presence of a biofilm-producing strain of *Staphylococcus epidermidis*.

Case report

A 77-year-old patient was referred to our clinic for evaluation of endophthalmitis secondary to an open globe injury from an olive tree branch that had occurred to his left eye 2 months earlier, at which time he had undergone primary repair with a monofilament 10-0 nylon suture. During the first hospitalization two injections of vancomycin (1 mg) were given in the anterior chamber. He was then discharged with both systemic (amoxicillin+clavulanic acid, 1 g twice daily) and topical antibiotic therapy: ofloxacin 0.3% eye drops (once hourly), tetracycline1%–sulfametoxazole 5% ointment (once daily). Atropine 1% eye drops (once daily) and timolol maleate 0.5% eye drops (twice daily) were also administered. At presentation to our clinic, his visual acuity was light perception OS. Examination of the left eye revealed mild upper and lower lid edema, chemosis, conjunctival hyperemia, hazy cornea with a 4-mm-diameter corneal ulcer with an inflammatory ring corneal infiltrate, and a 3-mm sclero–corneal linear wound apposed by a 10.0 nylon suture. The Seidel test was negative. A 4-mm hypopyon with fibrinous exudates was present in the anterior chamber. B-Scan ultrasound showed vitreitis with no sign of retinal detachment. There was no evidence of intraorbital or ocular foreign body on ultrasound or magnetic resonance imaging. Systemic intravenous piperacillin/tazobactam (4.5 g/day) was added to the initial therapy.

On the following day, specimens from the aqueous and vitreous chambers were obtained for microbiological examination, and the anterior chamber was irrigated with 1 mg of vancomycin. Two days postoperatively recurrence of inflammation was noted, with hypopyon formation. Due to the persistence of inflammation 9 days after hospitalization, the corneal suture was removed and sent for microbiological examination. A second injection of vancomycin was given into the anterior chamber. His clinical condition slowly improved, and the hypopyon slowly decreased. Seven days after suture removal all signs of inflammation had resolved.

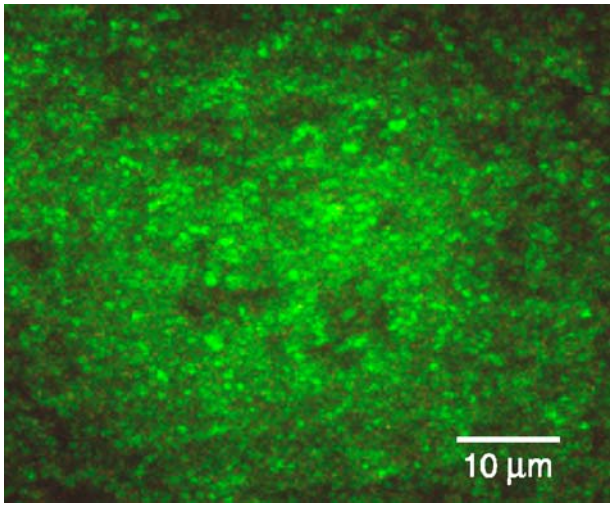

Fig. 1 Confocal scanning laser micrograph of microcolony formation in the early stages (8 h) of biofilm formation by the *S. epidermidis* strain obtained from the suture. Adsorbed bacteria cover most of the surface, in a thin monolayer, but distinct tower-like microcolonies, 15–20 µm high, have developed in some areas. Leica image reconstruction software [7]

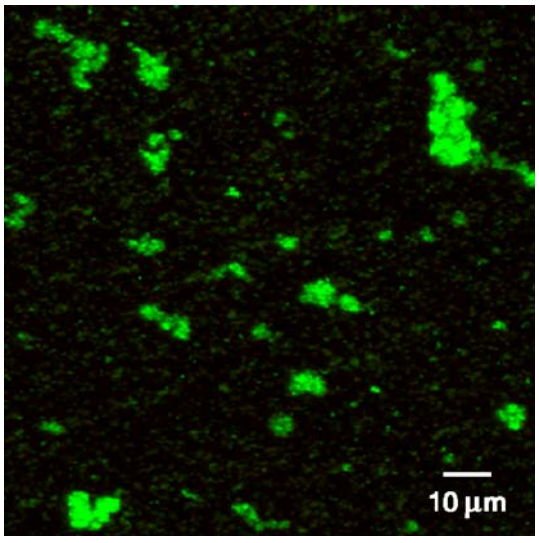

Fig. 2 Confocal scanning laser micrograph of a fully developed biofilm (4 days) formed by *S. epidermidis* obtained from the suture. This mound-like microcolony had a height of approximately 30 µm and a very high cell density. Leica image reconstruction software [7]

Eighteen months later, there were no signs or symptoms of infection.

A single strain of *S. epidermidis* was isolated [8, 9] both from aqueous and vitreous specimens and from the suture. In order to determine the propensity of the organism for growth in biofilms, it was grown at the Centre for Biofilm Engineering in a petri dish under continuous agitation, and a confocal scanning laser microscope (TCS NT; Leica Microsystems, Exton, PA, USA) was then used to analyze the samples [2]. Under these conditions, individual cells adhered to glass surfaces within the apparatus and formed microcolonies (Figs. 1 and 2) in a pattern very similar to that of *S. epidermidis* isolates from other device-related infections [4]. As biofilm formation proceeded, the sessile cells were surrounded by large amounts of matrix material and the microcolonies assumed the "tower" structures characteristic of most bacterial biofilms [3]. Three days after the initiation of biofilm formation, the individual microcolonies projected 30 μm from the colonized surface and were separated by the well-defined water channels characteristic of mature bacterial biofilms [3]. The results of antibiotic susceptibility tests [8, 9] showed (Table 1) that the isolated strain was susceptible to the antibiotics administered in this case when the organism was cultured in planktonic form. On the other hand, the isolate growing in biofilms on colonized surfaces was resistant to concentrations of the antibiotics much higher than those that killed planktonic cells of the same organism, and much higher than those that can be achieved, locally, with conventional antibiotic therapy.

Table 1 Minimum inhibitory concentration (*MIC*) and minimum bactericidal concentration (*MBC*) values of the isolated *S. epidermidis* strain (beta lactamase +), in both planktonic and sessile form

Antibiotic	Planktonic *S. epidermidis*		Biofilm	
	MIC	MBC	MIC	MBC
Vancomycin	3.12	3.12	**6.25**	**25**
Trimethoprim/sulfamethoxazole	0.39	25	**50**	**50**
Oxacillin	3.12	6.25	3.12	**25**
Tazobactam	0.78	1.56	0.39	**3.12**
Amoxicillin/clavulanic acid	0.78	1.56	0.78	**3.12**
Cefalotine	12.5	25	**12.5**	**50**
Cefapime	6.25	6.25	**25**	**50**
Ceftriaxone	6.25	12.5	6.25	**50**
Imipenem	3.12	3.12	6.25	**50**
Chloramphenicol	50	50	50	100
Gentamycin	3.12	6.25	3.12	**50**
Clarithromycin	0.78	3.12	1.56	**25**
Meropenem	3.12	6.25	3.12	**25**
Ciprofloxacin	0.19	0.19	0.19	**12.5**
Norfloxacin	0.19	0.19	0.39	**12.5**
Clindamycin	0.39	1.56	0.39	**3.12**
Nitrofurantoin	50	50	50	50
Erythromycin	1.56	6.25	3.12	**25**
Rifampin	1.56	6.25	1.56	**12.5**
Teicoplanin	3.12	3.12	3.12	**6.25**
Tetracycline	3.12	6.25	3.12	6.25
Penicillin	25	50	25	**100**

The results demonstrate that only the biofilm form of the same strain is resistant to antibiotics (data are expressed as micrograms per milliliter). In the majority of drugs considered MIC and MBC values are higher in the biofilm form (highlighted in bold) than in the planktonic form. All of these increased MIC and MBC levels are much higher than can be achieved in human therapy

Discussion

A case of posttraumatic endophthalmitis is described. This infection was unresponsive to specific and aggressive antibiotic therapy until the suture that served as the nidus of biofilm formation was removed. The strain of *Staphylococcus epidermidis* that was recovered from the suture, and from aqueous and vitreous specimens, was shown to form exuberant biofilms in vitro and these sessile communities were shown to be highly resistant to antibiotics, including those used to treat this infection. This case demonstrates that bacterial biofilm formation on even the most trivial of medical devices, like a single suture, can pose a danger that calls for vigilance in early detection of infection and for aggressive use of appropriate antibiotics following the removal of the device. This is the first clinical case in which microbiological studies of a surgical suture document the presence of a slime-producing strain of *S. epidermidis* with the capability of forming antibiotic-resistant biofilms.

The simple classification of suture-related eye infections as biofilm infections will have favorable consequences since device manufacturers are engaged in a concerted program to develop coatings and materials that delay or prevent biofilm formation. Some of these new materials are formulated to contain and release conventional antibiotics that kill the "incoming" bacteria while they are still planktonic, and some of these coatings have been successful in the perioperative time frame. Recent developments in device coatings involve the use of new agents that inhibit cell-to-cell signalling in bacteria. Some of these agents specifically inhibit biofilm formation by *S. epidermidis* [1, 5], the most common bacterial pathogen that affects medical devices that traverse the skin or the conjunctiva. If we can establish that a single monofilament suture can serve as nidus of infection, this knowledge will lead ophthalmic surgeons to use antibiofilm strategies in order to avoid these unnecessary complications of what should be routine surgery.

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