Biofilm formation and its effect on the management of culture-positive bacterial endophthalmitis

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Purpose: To compare the clinicomicrobiological features and outcomes in patients with infectious endophthalmitis caused by biofilm-positive (BP) and biofilm-negative (BN) bacteria.

Methods: This was a prospective, interventional, comparative, nonrandomized, consecutive case series. Culture-positive bacterial endophthalmitis cases from August 1, 2018 to July 31, 2019 were included. All vitreous samples were tested for biofilm using crystal violet plate and XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) methods and classified as BN and BP. The antibiotic susceptibility of all organisms was determined. Anatomic and functional success was defined as intraocular pressure ≥5 mm Hg and final best-corrected vision ≥20/400, respectively, at last visit. Results: There were 50 eyes in the BN group and 33 eyes in the BP group. BN group eyes required 2.86 ± 1.45 surgical interventions, and BP group eyes needed surgical interventions 6.36 ± 2.89 interventions, P < 0.0001, 95% Confidence Interval, CI: 0.4–1.7. Functional success was achieved in 44% and 21.2% (P = 0.03, 95% CI: 1.86%–40.08%) and anatomic success was achieved in 68% and 42.42%, respectively (P = 0.02, 95% CI: 3.85%–45.47%). The antimicrobial resistance patterns between the two groups were comparable.

Conclusion: Endophthalmitis caused by the biofilm-forming bacteria needs a greater number of surgical interventions. The anatomic and functional outcomes are poorer than non-biofilm-forming bacterial endophthalmitis. The increased virulence and poorer outcomes can be hypothesized to be due to the physical barrier effect of the biofilm on the antibiotics.

Key words: Bacterial endophthalmitis, biofilm, clinical outcome, endophthalmitis

Endophthalmitis is defined as inflammation of the inner coats of the eyeball, primarily involving the vitreous. It is one of the most dreaded eye conditions. Most cases occur either following surgery or trauma. The organisms gain entry either from exogenous sources such as trauma or surgery or from endogenous hematogenous spread from a distant site.[¹] Bacterial endophthalmitis is more common than fungal endophthalmitis. Staphylococcus epidermidis is the most common isolate in the USA,[²] Europe,[³] and India.[⁴]

Biofilm is one of the major causes of resistance to various antibiotics in systemic diseases.[⁵] Structurally, a biofilm is a slimy layer of an extracellular matrix made of polymeric substances produced by microorganisms [Fig. 1]. This forms an architectural colony providing resistance not only against antibiotics but also against the human immune system.[⁶] Role of biofilm has been studied in several ocular conditions where implants are used (such as intraocular lens, scleral buckles, punctal plugs, and lacrimal intubation devices) and not used (such as keratitis, chronic dacryocystitis, and endophthalmitis).[⁶] Microorganisms produce biofilm by various mechanisms related to biochemical, molecular, and altered host factors.[⁷] Leid et al.[⁸] demonstrated the development of histologically proven biofilm on the posterior surface of the lens capsule approximately 72 h after injecting 5000 cfu/ml of S. aureus RN 6390 into the mid-vitreous cavity in a murine model.

There are no reports on the impact of biofilm on the clinical management of endophthalmitis and its outcome in the existing literature. In the current communication, we present our results of evaluating bacterial biofilm and its role in the management outcome of endophthalmitis.

Methods

This was a prospective, comparative, nonrandomized, consecutive case series. Patients diagnosed with culture-positive bacterial endophthalmitis from August 1, 2018 to August 1, 2019 were recruited in the study. Data of patients with a minimum follow-up of 3 months were analyzed. The study was approved by the Institutional Review Board (LEC-7-18-118) and adhered to all the tenets of the Declaration of Helsinki on treating human subjects. Written informed consent was obtained from all patients, and from guardians when patients were younger than 18 years. The exclusion criteria included all culture-negative cases of infectious endophthalmitis, all cultures positive for fungus, and patients not consenting to the study.

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was recorded in terms of reduction of vitreous echoes on B-scan intravitreal injections were repeated every 48 hours. Media clarity were left to the decision of the treating physicians. In principle, plana vitreous lavage depended on the response to treatment and procedures such as repeat intravitreal antibiotics or repeat pars ciprofloxacin 750 mg two times per day for 7–10 days. Additional corticosteroid (prednisolone acetate 1% one hourly) and oral ciprofloxacin 750 mg two times per day for 7–10 days.

All patients underwent a detailed, comprehensive ophthalmic evaluation, including uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA) using Log MAR chart, slit-lamp biomicroscopy, intraocular pressure (IOP) by Goldman application tonometry (GAT), a dilated fundus examination using 78/90/20D lens, and B-scan ultrasound evaluation when fundus was not visible. Endophthalmitis was diagnosed clinically (and with B-scan ultrasonography when required) from a combination of symptoms (pain, red eye, lid edema, and reduced presenting vision) and signs (corneal edema, exudates in the anterior chamber, hypopyon, vitreous exudates, medium to high reflective dot or membranous opacities in the vitreous cavity, and a thickened chorioid).

Undiluted vitreous was processed in microbiology laboratory for direct microscopy and culture for bacteria and fungi as per the institutional protocol. The bacterial isolates were identified using the Vitek 2 compact system (bioMerieux), and antibiotic susceptibility was tested by a combination of E test and Vitek 2 for minimum inhibitory concentration (MIC) of several antibiotics and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The bacterial isolate was then tested for biofilm formation in vitro using the crystal violet method and XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) method.

Functional success was defined as visual acuity logMAR 1.3 (Snellen ≥20/400) and anatomical success as IOP >8 mm Hg.

Clinical management protocol

As per institute protocol, the surgical management of endophthalmitis consisted of pars plana vitrectomy, microscopy and culture of undiluted vitreous, antimicrobial susceptibility testing of bacterial isolates, and intravitreal antibiotics (vancomycin (1 mg/0.01 ml) + ceftazidime (2.25 mg/0.01 ml)) with or without dexamethasone (400 µg/0.01 ml). The medical treatment also included intensive topical antibiotics (ciprofloxacin 0.3% one hourly) and corticosteroid (prednisolone acetate 1% one hourly) and oral ciprofloxacin 750 mg two times per day for 7–10 days. Additional procedures such as repeat intravitreal antibiotics or repeat pars plana vitreous lavage depended on the response to treatment and were left to the decision of the treating physicians. In principle, intravitreal injections were repeated every 48 hours. Media clarity was recorded in terms of reduction of vitreous echoes on B-scan at each subsequent visit and in terms of improvement in the visibility of the retina and retinal vessels. Repeat injections were discontinued once media clarity increased to at least second-order retinal vessels visible. In cases with hazy view due to corneal involvement, a vitreous biopsy was taken instead of a vitrectomy procedure. The topical and intravitreal antibiotics used were adjusted as per the culture sensitivity report.

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Statistical methods

The collected data was arranged on an excel spreadsheet. Statistical analysis was analyzed using the MedCalc Statistical Software version 19.7.2 (MedCalc, Ostend, Belgium). All continuous data were classified as either normative or non-normative in each group. Paired t test was used to compare normative data, and the Mann–Whitney U test was used to compare nonnormative data. Analysis of categorical data was done using the Chi-square test. Odd’s ratio was calculated wherever appropriate. Multivariate logistic regression analysis was done to assess the effect of various demographic and clinical factors on the final anatomic and functional outcome. P < 0.05 was assigned as statistically significant.

Results

Eighty-three patients satisfied the inclusion criteria in the study period of 1 year. Fifty patients were biofilm positive, and 33 patients were biofilm negative with either CVP or XTT method or both. Demographic details of the patients in both groups are presented in Table 1. There was no statistical difference in the presenting age, duration of vision loss, or...
Table 1: Comparative presentations and outcomes of biofilm-negative and biofilm-positive organisms

|                          | Biofilm-negative | Biofilm-positive | P       | 95% C.I. for the difference |
|--------------------------|------------------|------------------|---------|----------------------------|
| Number of eyes           | 50               | 33               |         |                            |
| Males (%)                | 28 (56)          | 26 (78.8)        | 0.03    | 1.86%-40.08%               |
| Mean age in years (median)| 44.08±24.67 (50.5)| 46.51±19.24 (49)| 0.96    |                            |
| Duration of vision loss in days (median) | 4.48±5.63 (2.5) | 4.83±7.44 (2) | 0.45    |                            |
| Post cataract surgery (%) | 21 (42)          | 13 (39.4)        | 0.81    |                            |
| Presenting vision in logMAR (median) | 2.79±0.72 (3.3) | 2.72±1.13 (3.3) | 0.69    |                            |
| Glaucoma at presentation | 16 (32%)         | 10 (30.3%)       | 0.87    |                            |
| Vitreous tap as first procedure | 12 (24.5%) | 7 (22.6%)       | 0.84    |                            |
| Gram-negative etiology   | 24 (48%)         | 17 (51.51%)      | 0.75    |                            |
| Mean number of surgical interventions (median) | 2.86±1.45 (3) | 6.36±2.89 (6) | <0.0001 | 2-4                       |
| Follow-up in months (median) | 6.96±5.16 (6) | 5.8±4.74 (5) | 0.33    |                            |
| Final vision in logMAR (median) | 1.2±0.57 (1.2) | 2.17±0.88 (1.9) | 0.0005 | 0.4-1.7                   |
| Functional success       | 22 (44%)         | 7 (21.2%)        | 0.03    | 1.86%-40.08%               |
| Anatomic success         | 34 (68%)         | 14 (42.42%)      | 0.02    | 3.85%-44.47%               |

Discussion

The current study discusses the role of biofilm in the treatment outcome of bacteria endophthalmitis. It was noted that bacteria with in vivo biofilm formation in endophthalmitis showed enhanced in vivo virulence, leading to an increased need for surgical intervention and reduced final anatomic and functional success. The current study also included cases that were post-trauma. Trauma by itself is a confounding factor that can directly affect final clinical outcomes irrespective of the severity of endophthalmitis. However, as the number of cases post-trauma in both groups was comparable (P = 0.82), the effect of this confounding variable was avoided. Griffiths et al.[13] reported biofilm formation on an IOL by S. epidermidis in 1989. Adherence of the bacteria to the lens with glyocalyx around it prevents both antibiotics and antibodies from reaching the bacteria. Increased adherence of S. epidermidis to intraocular polypropylene lenses compared to polymethylmethacrylate lenses is known.[14] It is proposed that biofilm production on IOLs after cataract surgery occurs due to the microorganism’s ability to adhere to these lenses via exopolysaccharides produced by them. This provides an extra shell and protects these bacteria from the antibiotics.[15,16] Need for multiple interventions and eventually the IOL explantation has also been reported in acute and chronic biofilm-producing Staphylococcal endophthalmitis.[17] However, biofilm formation is not always associated with antibiotic resistance but may affect virulence. Biofilm producing S. epidermidis produces extracellular polymeric substances and inhibits phagocytosis and antibiotics’ action, resulting in inadequate clearance of organisms.[18] Thus, in these situations, multiple interventions may be required to sterilize the vitreous cavity. The current study showed the need for significantly more interventions in biofilm-producing microorganisms causing endophthalmitis as compared to those that were biofilm-negative.

Biofilms have been known to have a virulent role in ophthalmology, especially in cases where some kind of implant has been used for management of the disease. A study conducted by Holand et al.[19] demonstrated biofilm in 65% of scleral buckles (solid silicone and sponge forms) removed for infection and extrusion by scanning electron microscopy. Biofilms on scleral buckles may function as reservoirs for pathogenic bacteria contributing to its extrusion.[20] A report by Yokoi et al.[21] showed association of biofilm in punctal...
plugs developing conjunctivitis that required removal of the plug and prolonged antibiotic treatment. Periorbital implants include orbital plates, porous polyethylene floor implant, orbital sphere implants, and anophthalmic socket sphere implants or metal screws. Scanning electron microscopy has demonstrated polymicrobial or mixed species biofilm on these implants. However, most commonly seen organism in orbital implants was *S. aureus*. Other organisms included *M. chelonae* and *Pantoaea agglomerans* found in polymicrobial cases, yeasts (*Candida* spp. and *Trichosporon* spp.), *Staphylococcus* spp., *M. chelonae*, and gram-negative bacilli (*Achromobacter xylosoxidans* and *P. aeruginosa*) in orbital plates.[22] Virulence related to biofilm formation has also been proposed in contact lens-related keratitis. One of the commonest organisms implicated in contact lens-associated keratitis is *Pseudomonas* species, which is also known to form biofilm. A study conducted by Abidi et al.[23] showed that all species of *Pseudomonas* were found to be potential biofilm formers and also concluded that the multidrug-resistant isolates displayed significant biofilm production as compared to susceptible isolates, indicating the antimicrobial resistance offered by the biofilm to these organisms.[23] Biofilm also forms with *Acanthamoeba* spp., which is another common organism involved in contact lens-associated keratitis.[24]

The current study has its strengths and limitations. Among strengths, this was a prospective consecutive case series where the patients were treated by a uniform institutional protocol, but the treating physicians were masked to the results of the biofilm. Among limitations, the biofilm formation was tested in vitro in cultures and not over IOLs because its exploitation was not needed in any of the patients. Thus, our study is an in vivo extrapolation of the in vitro observation. Furthermore, the cases in this study included etiologies other than intraocular surgery, such as open globe injury. This makes the instances

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### Table 3: Microorganisms isolated in the biofilm-positive and biofilm-negative groups

| Microorganism          | Biofilm-negative | Biofilm-positive |
|------------------------|------------------|------------------|
| **Gram-positive**       |                  |                  |
| *Streptococcus* species| *S pneumoniae*   | 7                | 1                |
|                        | *S anginosus*    | 1                | -                |
|                        | *S pyogenes*     | 1                | -                |
|                        | *S mitis*        | 1                | -                |
|                        | *S licheniformis*| 1                | -                |
|                        | *S gordonii*     | -                | 1                |
|                        | *S agalactia*    | -                | 1                |
| *Staphylococcus* species| *S epidermidis*  | 6                | 9                |
|                        | *S aureus*       | 4                | 1                |
|                        | *S lugdunensis*  | -                | 1                |
| *Bacillus* species      | *B cereus*       | 4                | 1                |
|                        | *B licheniformis*| 1                | -                |
| *Gemella* species       | *G morbillorum*  | -                | 1                |
| **Total. n=42**         |                  | 26 (61.9%)       | 16 (38.1%)       |

| **Gram-negative**       |                  |                  |
| *Pseudomonas* species   | *P aeruginosa*    | 10               | 8                |
|                        | *P stutzeri*      | -                | 3                |
| *Stenotrophomonas* species| *S maltophilia*  | 2                | -                |
| *Aeromonas* species     | *A hydrophila*    | 1                | -                |
| *Brevundimonas* species | *B vesicularis*   | 1                | -                |
| *Escherichia* species   | *E Coli*          | 1                | -                |
| *Acinetobacter* species | *Alwofii*         | 1                | -                |
| *Klebsiella* species    | *K pneumoniae*    | -                | 2                |
| *Enterobacter* species  | *E aerogenes*     | -                | 1                |
|                        | *E cloacae*       | 7                | 1                |
| *Serratia* marcescens   | *S marcescens*    | -                | 1                |
| *Cronobacter* sakazakii | *C sakazakii*     | -                | 1                |
| *Pantoea* species       | 1                | -                |                  |
| **Total. n=41**         |                  | 24 (58.5%)       | 17 (41.5%)       |

### Table 4: Comparison of antibiotic resistance patterns between the two groups

| Antibiotic tested for resistance | Biofilm-negative | Biofilm-positive | *P* |
|----------------------------------|------------------|------------------|-----|
| *Vancomycin*                     | 4.52% (n=22)     | 10% (n=10)       | 0.55|
| *Ceftazidime*                    | 60.86% (n=23)    | 33.33% (n=15)    | 0.1 |
| *Ciprofloxacin*                  | 31% (n=29)       | 35.29% (n=17)    | 0.76|
| *Amikacin*                       | 52.63% (n=19)    | 40% (n=15)       | 0.47|
| *Chloramphenicol*                | 20% (n=45)       | 34.48% (n=29)    | 0.16|
| *Imipenem*                       | 66.66% (n=15)    | 30% (n=10)       | 0.07|
heterogeneous, but a logistic regression analysis did not impact the etiological differences on the final clinical outcome.

**Conclusion**

In conclusion, we propose that early identification of biofilm-forming organisms may help decide a tailored management strategy for each patient. It would also help in proper prognosticating the outcome. These results can serve as a background for further research into anti-biofilm measures impacting clinical outcomes in patients with endophthalmitis.

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**Conflicts of interest**

There are no conflicts of interest.

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