

In vitro study on the effect of antibiotic combinations on *Staphylococcus epidermidis* biofilms

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Abstract

Purpose: Effect of combination of various antibiotics on *Staphylococcus epidermidis* biofilm.

Study design: Experimental study.

Methods: The biofilm-producing strains of *S. epidermidis* were isolated from 100 patients undergoing cataract surgery before instillation of any antibiotic. The strains were subjected to sensitivity test to various antibiotic combinations. The most effective agent was selected and its minimum inhibitory concentration was determined by broth dilution method. The statistics were performed using SPSS Version 23 (IBM Corp.) and Chi square test.

Results: A total of 22 biofilm-positive samples were obtained. The combinations of vancomycin with ceftazidime ($p < 0.05$) followed by moxifloxacin with cefuroxime ($p < 0.05$) were found to be the most effective. Antibiofilm activity was also shown by other antibiotic combination. The minimum inhibitory concentration of vancomycin and ceftazidime in 11 samples was 2.5 and 2.8 mg/ml, while in the rest of the samples, it was 2.5 and 5.6 mg/ml, respectively. The minimum inhibitory concentration of moxifloxacin and cefuroxime was 0.125 and 2.81 mg/ml, respectively.

Conclusion: In our study, we conclude that antibiotics are effective in eradicating biofilms.

Keywords: biofilms, ceftazidime, cefuroxime, endophthalmitis, moxifloxacin, *Staphylococcus epidermidis*, vancomycin

Introduction

The postoperative endophthalmitis is the most devastating complication after cataract surgery. The reported incidence varies from 0.01 to 0.367%, and it differs among various surgeries, across studies and countries. It is reported

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that 70% of postoperative endophthalmitis occur following cataract surgery. Coagulase-negative *Staphylococcus epidermidis* bacteria has been recognized as the most common organism isolated from the cases of postoperative endophthalmitis.¹ *S. epidermidis* is the main commensal bacterium found on human skin, conjunctiva, and eyelids. It is nowadays seen as an important opportunistic pathogen and causative agent for nosocomial infections, similar to *Staphylococcus aureus* (Centers for Disease Control and Prevention, National Nosocomial Infection Surveillance, 2004). *S. epidermidis* possesses high affinity for indwelling medical devices. The pathogenicity of *S. epidermidis* is contributed by formation of biofilm.² Bacteria use biofilm as a way to survive in the nature and hence are difficult to treat because of resistance to host defence and antibiotics.^{3,4} The resistance can be contributed to complex biofilm structure and its role in adherence to inert surface.⁵ In our study, we tried to select best antibiofilm agent to prevent the postoperative endophthalmitis in patients undergoing cataract surgery. We compare the effect of combination of vancomycin and ceftazidime, vancomycin and amikacin, vancomycin and tobramycin, and moxifloxacin and cefuroxime. Vancomycin is a glycopeptide and covers gram-positive bacteria, whereas ceftazidime and aminoglycosides like tobramycin and amikacin cover gram-negative bacteria. It has been shown in various studies that bacterial contamination occurs during implantation of intraocular lenses (IOLs) and it can be prevented by antibiotic prophylaxis. The aim of our study was to see various antibiotics in combination on the inhibition of biofilm by *S. epidermidis* in patients undergoing cataract surgery and hence prevention of occurrence of postoperative endophthalmitis.

Materials and methods

This study was an experimental study done in 24 months in Institute of Ophthalmology, Jawaharlal Nehru Medical College Hospital (JNMCH), AMU, Aligarh, from October 2015 to September 2017.

Bacterial strains

The bacterial strains of *S. epidermidis* were isolated from conjunctival sac and lids of the 100 patients undergoing cataract surgery in JNMCH, AMU, Aligarh. The biofilm producer *S. epidermidis*—MTCC 435 (Microbial Type Culture Collection) and non-biofilm producer *S. epidermidis*—ATCC 12228 (American Type Culture Collection) were used as controls.

Media used in the study

Nutrient agar for the initial isolation of *S. epidermidis* strains and to determine morphological characteristics of the bacteria.

Modified Congo red agar (CRA) to differentiate the strains of biofilm producers from non-biofilm producers.

Agents used

1. Inj. vancomycin = 1 mg in 0.1 ml
2. Inj. ceftazidime = 2.25 mg in 0.1 ml
3. Inj. tobramycin = 0.2 mg in 0.1 ml
4. e/d or single moxifloxacin = 0.5% in 0.4 ml. Take 0.05 ml directly (0.0025 mg in 0.05 ml)
5. Inj. cefuroxime = 1 mg in 0.1 ml

IOL used

The polymethylmethacrylate (PMMA) IOLs were used in the study. The IOLs were used because their surface acts as a platform for the formation of biofilm.

Technique

The conjunctival and lid swabs obtained during the study were seeded on nutrient agar and stained by Gram staining for determination of purity and morphology and specific staining for isolation of *S. epidermidis* strains. After confirmation of these cultural and morphological characteristics, the strains were subjected to catalase and coagulase tests and other relevant biochemical tests for further identification. The positive growth was transferred to nutrient broth.

Study of biofilm production

The phenotypic characterization of biofilm production was performed by modified CRA plates as proposed by Kaiser *et al.*⁶ The biofilm producers formed black colonies and the non-biofilm producers formed red colonies.

Antibacterial susceptibility examination of agents under study

The biofilm-producing strains of *S. epidermidis* were subjected to inhibition studies using different antibiotics in combination. For susceptibility, disc diffusion method was used and zone of inhibition was interpreted by NCCLSM100-S12. The media used was nutrient agar. The bacterial suspension of the titrated strain that gives 100 CFU was selected for inoculation on culture plate for antibiotics. After overnight incubation, the zone of inhibition was observed and measured by calipers or ruler. The results are recorded in millimetres, and accordingly, the most effective drug will be selected on the basis of inhibition zone.

Agents used for antibiotic inhibition

Vancomycin and ceftazidime, vancomycin and amikacin, vancomycin and tobramycin, moxifloxacin and cefuroxime.

The most effective agent was selected and its minimum inhibitory concentration was determined by broth dilution method. About 1 ml of bacterial inoculum and 1 ml of antimicrobial agent were taken in the test tubes along with IOLs and were incubated at 37°C for 24 hours. The antibiotic concentration in least turbid inoculum was taken as minimum inhibitory concentration. The positive control was kept for comparison and it showed growth button. After 24 hours, lenses were taken out and each IOL was gently washed with phosphate-buffered saline and stained with 1% crystal violet to stain and detect biofilm.

Statistical tests

Analysis was done by using IBM SPSS version 23 and Chi square test.

Results

A total number of 100 patients undergoing cataract surgery, 52 males and 48 females, were included in this study. The male-to-female ratio was found to be 1:1 (male = 52, female = 48). The mean age of male and female was 58.53 ± 11.74 and 60.31 ± 11.56 years, respectively. All patients were admitted for cataract surgery with IOL implantation. The eye specimens were obtained with the help of moistened sterile cotton swabs in order to isolate *S. epidermidis* strains among

Table 1. Screening of *S. epidermidis* isolates for biofilm production on modified CRA medium

S. no.		Biofilm-positive isolates	Biofilm-negative isolates	Total isolates
1.	Number of isolates	22	12	34
2.	Percentage of isolates	64.71	35.29	

Table 2. Incidence of sensitivity and resistance of biofilm-positive strains to various antibiotic combinations

Drug	Susceptible no (%)	Resistant no (%)	p value
Vancomycin and ceftazidime	18 (81.8)	4 (18.2)	<0.05
Vancomycin and amikacin	12 (54.5)	10 (45.5)	>0.05
Vancomycin and tobramycin	13 (59.09)	9 (40.9)	>0.05
Moxifloxacin and cefuroxime	16 (72.7)	6 (27.3)	<0.05

the normal conjunctival and periocular flora.

Among the confirmed *S. epidermidis* isolates, the percentage of biofilm-positive isolates was found to be 64.71 and the percentage of biofilm-negative isolates was 35.29, as determined by the modified CRA method (Table 1).

As shown in Table 2, the 18 of 22 strains were sensitive to vancomycin and ceftazidime and it was significant ($p < 0.05$). The sensitivity to combination of vancomycin and amikacin was shown by 12 strains whereas about 13 strains were sensitive to vancomycin and tobramycin and it was not significant ($p > 0.05$). The 16 strains showed sensitivity to moxifloxacin and cefuroxime and was significant ($p < 0.05$). Out of 22 isolates of *S. epidermidis*, combination of vancomycin and ceftazidime was the most effective followed by combination of moxifloxacin and cefuroxime. The minimum inhibitory concentration was determined by standard broth dilution method as advised by Clinical and Laboratory Standards Institute (CLSI). The minimum inhibitory concentration of vancomycin and ceftazidime in 11 samples was 2.5 and 2.8 mg/ml, respectively, and in rest of the samples, it came out to be 2.5 and 5.6 mg/ml, respectively. The minimum inhibitory concentration of moxifloxacin and cefuroxime was 0.125 and 2.81 mg/ml, respectively.

Discussion and conclusion

This study was undertaken to find the role of various antibiotics in prevention of *S. epidermidis*-producing biofilms on IOLs and is first of its kind, thereby highlighting the importance of prophylaxis against endophthalmitis in patients undergoing cataract surgery. We had already conducted study on the adhesion of biofilm-forming *S. epidermidis* strains on IOLs.⁷ They compare biofilm formation and adhesion on different IOL materials by *S. epidermidis* isolates obtained from cataract surgery patients.⁸ Bacterial adherence to the implant surfaces and biofilm formation seem to depend on the hydrophobicity or hydrophilicity of the biomaterial. The results of this *in vitro* study suggest that *S. epidermidis* biofilms form more readily on hydrophobic acrylic IOLs and least on silicone IOL material. The best possible IOL material, therefore, in terms of suitability for implantation in cataract surgery seems to be silicone compared with PMMA or hydrophobic acrylic (silicone > PMMA > hydrophobic acrylic) in order to minimize biofilm formation and adhesion by *S. epidermidis* strains. The PMMAs were used as these are the most widely used in our institute. So this study is a step forward from the previous study. This study was an attempt to find the best possible agent effective against the biofilm formation on IOLs.

The strains of *S. epidermidis* are supposed to constitute the normal flora of eye but it may be pathogenic and can lead to devastating infections, as mentioned in Medical Microbiology, 4th ed., by Davis (1996). A number of researchers have carried out various studies to determine the incidence of *S. epidermidis* strains, including

methicillin-resistant *S. epidermidis* (MRSE) among preoperative cataract surgery patients. Olson *et al.* performed a study on preoperative cataract patients to find characteristics of bacterial flora on ocular and periocular surfaces. The incidence of *S. epidermidis* was 62.9%, followed by *S. aureus* (14.0%). MRSE accounted for 47.1% of *S. epidermidis* isolates, and methicillin-resistant *S. aureus* accounted for 29.5% of *S. aureus* isolates.⁹ In another study in Taiwan, the researchers collected the conjunctival and nasal culture from patients undergoing cataract surgery before instillation of any antibiotic solution. The most common organism found was coagulase-negative *Staphylococcus*.¹⁰ The incidence of *S. epidermidis* was 34% in the patients undergoing cataract surgery in this study.

The most common organism found in the patients of endophthalmitis after cataract surgery, according to the European Society of Cataract and Refractive Surgeons (ESCRS), was *S. epidermidis* (33-77%), followed by *S. aureus* (10-21%). The study of normal conjunctival flora by Keshav and Basu¹¹ found the same results. Similarly, Trinavarat and Atchaneeyasakul¹² investigated the eyes of postoperative endophthalmitis. The most common bacteria found were coagulase-negative *Staphylococci* and the other were *Streptococcus* spp. and *Corynebacterium* spp. In light of our research, we found that incidence of coagulase-negative *Staphylococci* was 34%, which is a high incidence and this may lead to serious IOL-related infections like endophthalmitis. These results are similar to the Endophthalmitis Vitrectomy Study (EVS) done by ESCRS and the study done by Trinavarat and Atchaneeyasakul in 2005.

The screening of confirmed *S. epidermidis* isolates for the production of biofilm was done by modified CRA method, as described by Kaiser *et al.*⁶ with minor modifications. The method is easy to carry out and the results are usually based on the colony colour produced, which ranges from red for non-biofilm-producing strains to black for biofilm-producing strains. This method gave better visualization and easier interpretations, due to the colour homogeneity of the spots, especially for the biofilm-producing strains. Biofilm-forming bacteria develops different mechanism to resist the immune system and antimicrobial drugs.¹³ These bacteria alter gene expression, decrease penetration of drugs, and maintain their high density and slow growth.¹⁴ So these factors lead to antibiotic resistance and hence increased virulence.

Vancomycin is a glycopeptide and covers gram-positive bacteria whereas ceftazidime and aminoglycosides like tobramycin and amikacin cover gram-negative bacteria. It is obvious that bacterial adhesion on IOLs occurs during lens implantation phase in cataract surgery. A study done by Özkan *et al.*¹⁵ showed that cefuroxime (0.2 mg/ml), teicoplanin (0.1 mg/ml), and vancomycin (0.1 mg/ml) significantly inhibit bacterial adherence to IOLs. The effect of cefuroxime on adherence inhibition was significantly higher than that of teicoplanin and vancomycin. Bacterial adherence is an important factor in bacterial virulence.

Antibiotics, especially cefuroxime, can successfully inhibit bacterial adherence.

In an *in vitro* study in 2003, Drago *et al.*¹⁶ proved the role of tobramycin and chloramphenicol against the *Pseudomonas aeruginosa* and *S. aureus* biofilm on IOLs. We also use combination of vancomycin and tobramycin for inhibition of biofilm strains of *S. epidermidis*: In this study, this combination was able to inhibit 13 biofilm-positive strains. Vancomycin is active against gram-positive bacteria and used as first line of drug in endophthalmitis. As per EVS, vancomycin is safe to use at 1 mg/0.1 ml concentration. In another study, decrease in *S. epidermidis* biofilm formation on IOLs was noted after treating with vancomycin.¹⁷ Vancomycin was used in concentration of 1 mg/0.1 ml in this study for sensitivity, which is an intravitreal dose vancomycin as suggested by EVS group in 1995. According to Koul *et al.*¹⁸ reduction of endophthalmitis by cefuroxime can be contributed to its anti-adhesion effect. In ESCRS, five- to six-times decrease in endophthalmitis was observed after using the intracameral injection of cefuroxime (1 mg in 0.1 ml in normal saline) at the end of cataract surgery.¹⁹

Subconjunctival injection of cefuroxime could be beneficial as it shows sustained increase in anterior chamber concentration even after two hours of injection.²⁰ Moxifloxacin and gatifloxacin combination shows good ocular permeability and has less side effect.²¹ Barreau *et al.*²² showed decrease in the occurrence of endophthalmitis with intracameral cefuroxime from 2,289 patients compared without intracameral cefuroxime (35 from 2,826 patients) with statistically significant ($p < 0.0001$) difference.

The synergistic effect was observed by addition of amikacin and rifampin with vancomycin and teicoplanin on *S. epidermidis* biofilm eradication on polyvinyl catheters.²³ In our study, vancomycin (1 mg/0.1 ml) with amikacin (0.4 mg/0.1 ml) was able to show inhibitory effect on 12 biofilm-positive strains and remaining 10 strains showed resistance, and results were statistically insignificant ($p > 0.05$). The resistance can be contributed to selected subpopulations of *S. epidermidis* that may be methicillin resistant, reducing the effect of antibiotics on biofilm.²⁴

Singh *et al.* in 2009 found that *S. aureus* and *S. epidermidis* biofilms reduce the penetration of oxacillin, cefotaxime, and vancomycin whereas no effect was noted that of amikacin and ciprofloxacin. Thus, combination of vancomycin with aminoglycosides (amikacin and tobramycin) may give additive effect on the inhibition of biofilm production and leads to decreased incidence of postoperative endophthalmitis.²⁵

Karadag *et al.*²⁶ reported that moxifloxacin and cefuroxime effectively decrease the adhesion of bacteria to IOL surface. In 2016, Benbouzid *et al.*²⁷ compared the anti-adherence effect of cefuroxime (1 mg/0.1 ml) and moxifloxacin (0.5 mg/0.1 ml) on the primary attachment phase of *S. epidermidis* on hydrophobic acrylic IOLs.²⁷ Both moxifloxacin and cefuroxime were able to reduce *S. epidermidis* adhesion on

hydrophobic acrylic IOLs significantly. Moxifloxacin was more effective in preventing adherence. Haripriya et al.²⁸ concluded that routine moxifloxacin (0.5 mg/0.1 ml) prophylaxis leads to reduction of endophthalmitis by 3.5-fold (threefold for small-incision cataract surgery and nearly sixfold for phacoemulsification).

Bacterial virulence is the result of bacterial adhesion. Many researches have been done in the past years to know exact mechanism behind *S. epidermidis* biofilm. Antibiotics are found to be effective in biofilm prevention. So, prior use of antibiotics can halt endophthalmitis caused by biofilm. More studies should be conducted to inhibit the formation and prevention of biofilm.

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