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Original Research Article

Bacterial isolates and their biofilm formation on contact lenses

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ABSTRACT

Introduction: Using contact lenses without proper care and sterilization measures can lead to eye infection like keratitis and other eye diseases. About 25-30% of cases with corneal ulcers are related to contact lens use. Microbial agents after their entry, they further invade into corneal layers by their virulence factors and enzymatic secretions by the bacteria. Wearing contact lenses also increase stress on cornea so the cornea has less ability to defend against the bacteria which is invading. Some of the bacteria are also capable of biofilm formation. Biofilms formed on contact lens surfaces has increased resistance to common sterilants used in contact lens care solution.

Aims/Objectives: The main aim of this study is to identify the pathogens that can be grown on the contact lenses and contact lens cases, to identify the production of biofilm by the isolates obtained during this study and to bring awareness about proper sterilization of contact lenses and lens cases during their usage to prevent unnecessary eye infections.

Materials and Methods: This cross sectional study was carried from October 2019 to June 2021 on MBBS and BDS students in Konaseema Institute of Medical Sciences and Research Foundation, who are using contact lenses and who are asymptomatic. Written consent from all students participating in this study was taken. The samples are collected using sterile swabs. Swabs from both the lens and lens cases are taken and immediately cultured and related biochemical tests are done to identify the organism. For the study of biofilm formation, the bacterial isolates obtained from the specimen are inoculated on to commercially available Congo red agar (CRA).

Data analysis: The data was processed and arranged into distribution tables and cross tables using statistical package for the social sciences (SPSS) version 21.

Results: Total 200 samples were collected and processed for bacterial culture. Among them 145(72.5%) samples showed positive bacterial cultures. The most common organisms obtained are aerobic spore bearing bacilli (ASB) which were 30 (20.6%), next common organism isolated were micrococci 24(16.5%), though ASB's and micrococci are non pathogenic, these were included because the lens and lens cases are preferably to be sterile. The other organisms isolated are diphtheroids 21 (14.4%), Coagulase negative *Staphylococcus aureus* (CONS) 18 (12.4%), *Klebsiella species* 16 (11.04%), *Proteus species* 12 (8.2%), *Citrobacter* 10 (6.9%), *Escherichia coli* 8 (5.5%) and *Staphylococcus aureus* 6 (4.2%). The isolated bacteria are then again streaked on Congo Red Agar to check for the ability of the bacterial isolates to form biofilms. Among 145 bacterial isolates obtained in this study 98 (67.5%) samples showed positive biofilm formation.

Conclusion: The micro organisms can lodge in most of the contact lenses and contact lens cases. . . which in turn enter into eye due to improper sterilization of lens and lens cases. And most of the bacterial isolates obtained from these contact lenses and contact lens cases are capable of production of biofilm. To avoid infections caused by contact lens users the care of both the contact lenses and lens cases is very important.

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1. Introduction

Using contact lenses without proper care and sterilization measures can lead to eye infection like keratitis and other eye diseases.^{1,2} About 25-30% of cases with corneal ulcers are related to contacts lens use.³ Microbial agents after their entry, they further invade into corneal layers by their virulence factors and enzymatics secreted by the bacteria. Wearing contact lenses also increase stress on cornea so the cornea has less ability to defend against the bacteria which is invading. Because of these factors bacteria manages itself to overcome the eyes natural barrier system and could precipitate the infection.⁴ If the contact lens is not properly maintained with sterilization, the cases could also be contaminated and they could turn as the potential source of infection. Many times the pathogen isolated from the lens can also be isolated from its lens case.⁵⁻⁷ Contamination of contact lenses and eye infection generally occurs as a result of not following the instructions given by the ophthalmologists regarding steps to be followed during cleaning the lenses. Improper cleaning and handling of lens, using water to rinse the lens instead of the lens cleaning solutions, prolonged use, reuse and poor hygiene can cause contamination of lenses and their case and this leads to eye infections and keratitis²

Few studies recently shown that *Pseudomonas aeruginosa*, along with other strains of bacteria, can accelerate colonization of contact lens surfaces in the presence of dying neutrophils in vitro.^{8,9} Analysis of the microbial burden on the lens has been increased by the slime produced by the bacteria increased its virulence thereby showed both an increase in viable bacteria and thickness of the newly formed biofilm. Direct targeting of neutrophil–bacterial interactions during contact lens wear in vivo has been shown to reduce bacterial internalization into the cornea.⁸ In few studies It has been reported that biofilms formed on contact lens surfaces has increased resistance to common sterilants used in contact lens care solutions.¹⁰ Importantly, current commercial contact lens care solutions fail to fully remove cellular debris from contact lens surfaces using recommended rub and rinse cleaning practices. The residual debris may represent a new risk factor for microbial recolonization of contact lenses. A biofilm has been defined as a “functional consortia of micro-organisms, organized at interfaces, within exopolymer matrices”.¹¹ Biofilm protects microbes against, phagocytes, antibiotics and bacteriophages and hence help in their survival.¹² Both Gram positive and Gram negative bacteria possess the ability to form biofilm such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.¹³ Biofilms play a major role in

more than 80% of bacterial infection.^{14,15} Biofilms were observed on contact lenses, lens cases, intraocular lens, glaucoma tubes, stents, punctual plugs, scleral buckle, corneal sutures, or other ocular prostheses.^{6,10} Extracellular polymeric substances (EPS) are secreted by the bacteria that hold together heterogeneous mixtures of bacteria and therefore are an important component of biofilm production.¹⁶ The main components of EPS in *S. aureus* and *S. epidermidis* are Polysaccharide intercellular adhesion (PIA) and capsular polysaccharide/adhesin.^{1,15}

2. Aims/Objectives

The main aim of this study is to

1. Identify the pathogens that can be grown on the contact lenses and contact lens cases
2. To identify the production of biofilm by the isolates obtained during this study.
3. To bring awareness about proper sterilization of contact lenses and lens cases during their usage to prevent unnecessary eye infections.

3. Materials and Methods

This cross sectional study was carried from October 2019 to June 2021 on MBBS and BDS students in Konaseema Institute of Medical Sciences and Research Foundation, who are using contact lenses and who are asymptomatic. The students who regularly visit ophthalmology clinics for some othe eye problems like eye infection, styes and on some other ophthalmology treatment were excluded. This study includes only the students who are wearing contact lenses daily atleast 5hrs per day in their routine student life. Written consent from all students participating in this study was taken.

The samples are collected using sterile swabs. Swabs from both the lens and lens cases are taken and immediately cultured on Blood agar, Nutrient agar and MacConkey agar and incubated at 37°C for 24 to 48 hours and anaerobic conditions. The colonies were determined morphologically by Gram staining and cultured under specific culture media and the appropriate biochemical tests and specific standard microbial tests such as oxidase, catalase, and coagulase tests, indole, MR, VP, sugar fermentation and Amino acid decarboxylation tests were done to identify the organism.¹⁷ The sensitivity of bacteria to antibiotics was determined according to the method of Kirbybauer test.¹⁷ The susceptibility of the microorganisms was interpreted according to the standard breakpoints determined by the CLSI 2019

For the study of biofilm formation, the bacterial isolates obtained from the specimen are inoculated on to commercially available Congo red agar (CRA). Strongly positive Biofilm bacterial isolates showed black colonies with a dry crystalline consistency. Moderately positive

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biofilm bacterial isolates showed darkening of colonies without crystalline consistency. Non biofilm bacterial isolates remained pink colour. Lab confirmed biofilm producing strain is taken as positive control.

Before initiation of study, institutional ethics committee approval in KIMS RF is taken and also written consent of patients is also taken from all participants.

3.1. Data analysis

The data was processed and arranged into distribution tables and cross tables using statistical package for the social sciences (SPSS) version 21.

4. Results

Total 200 samples were collected and processed for bacterial culture. Among them 145 (72.5%) samples showed positive bacterial cultures as shown in Table 1. Which is statistically significant (pvalue <0.005). Some samples showed multiple organisms. The most common organisms obtained are *aerobic spore bearing bacilli (ASB)* which were 30 (20.6%), next common organism isolated were *micrococci* 24(16.5%), though ASB's and *micrococci* are non-pathogenic, these were included because the lens and lens cases are preferably to be sterile. The other organisms isolated are *diphtheroids* 21 (14.4%), *Coagulase negative Staphylococcus aureus (CONS)* 18(12.4%), *Klebsiella species* 16 (11.04%), *Proteus species* 12(8.2%), *Citrobacter* 10(6.9%), *Escherichia coli* 8(5.5%) and *Staphylococcus aureus* 6 (4.2%) as shown in Table 2.

The isolated bacteria are then again streaked on Congo Red Agar to check for the ability of the bacterial isolates to form biofilms figure1. Among 145 bacterial isolates obtained in this study 98 (67.5%) samples showed positive biofilm formation as shown in Table 3.

Among 30 ASB isolates 56.6% have produced biofilm on Congo Red Agar. Which is statistically significant (p value <0.05). 18(75%) out of 24 *micrococci* isolates, 13(62%) out of 21 *diphtheroid* isolates, 16(88.8%) out of 18 isolates, 16(100%) out of 16 *Klebsiella* isolates, 4(33.3%) out of 12 *Proteus* isolates, 4(40%) out of 10 *Citrobacter* isolates, 5(62.5%) out of 8 *Escherichia coli* isolates and 6(100%) out of 6 *Staphylococcus aureus* also produced biofilm on Congo Red Agar as shown in Table 4.

Table 1:

Culture results	Study group n= number of cases	P value
Positive culture	145 (72.5)	<0.005
Negative culture	55 (27.5)	<0.005
Total samples	200	

Table 2:

Type of isolate	Study group n= numbe of cases	Percentage
ASB'S	30	20.6%
<i>Micrococci</i>	24	16.5%
<i>Diphtheroids</i>	21	14.4%
CONS	18	12.4
<i>Klebsiella</i>	16	11.03
<i>Proteus</i>	12	8.2%
<i>Citrobacter</i>	10	6.9%
<i>Escherichia coli</i>	8	5.5%
<i>Staphylococcus aureus</i>	6	4.1%

Table 3:

Biofilm formation	Study group n= number of cases	P value
Positive	98	<0.05
Negative	47	<0.05
Total	145	

Table 4:

Organism	Number of organisms isolated	Positive for biofilm	Percentage of biofilms formed
ASB	30	17	56.6%
<i>Micrococci</i>	24	18	75%
<i>Diphtheria</i>	21	13	62%
CONS	18	16	88.8%
<i>Klebsiella</i>	16	16	100%
<i>Proteus</i>	12	4	33.3%
<i>Citrobacter</i>	10	4	40%
<i>Escherichia coli</i>	8	5	62.5%
<i>S.aureus</i>	6	6	100%



Fig. 1: a: Showing biofilm positive with black coloured colonies; **b:** Showing biofilm negative with red coloured colonies.

5. Discussion

Several authors reported that the introduction of contact lenses was associated with increase in ocular problems associated with microbial contamination.^{18,19}

The human eye structure has, constant exposure directly to the environment. The use of contact lenses has an impact of developing many infectious eye diseases caused by parasites, viruses and bacteria. Some of these infectious eye diseases, were very rare prior to the invention of contact lenses. There is increasing microbial eye infections when people started wearing contact lenses. Host defenses directed against these pathogenic microorganisms are decreased with the use of contact lenses.^{20,21} Therefore, necessary precautions are to be taken to protect the eye from these opportunistic organisms. These microorganisms and their pathogenic effects might be different from one place to other, particularly in the developing countries.²²

The bacterial isolates obtained from the samples collected from lens and lens cases are Aerobic spore bearing Bacilli, *Micrococci*, *Diphtheroids*, *Klebsiella*, *Proteus*, *Citrobacter*, *Escherichia coli* and *Staphylococci*. Which are commonly isolated in other studies like Michael et al,²³ Raksha et al,²⁴ Najia et al.²⁵

The most common organisms obtained are aerobic spore bearing bacilli (ASB) which were 30 (20.6%), which are also isolated in Najia et al study²⁵ which are about (6.3%). Next common organism isolated were *micrococci* 24(16.5%) which were also isolated in Raksha et al²⁴ study as highest isolated organism about 75%. The other organisms isolated are *diphtheroids* 21 (14.4%) which were also isolated in other studies like Raksha et al,²⁴ *Coagulase negative Staphylococcus aureus (CONS)* 18(12.4%) which were also isolated in Najia et al study (5.6%), Michael et al²³ 8.5% and Raksha et al study 23%, *Klebsiella* Species isolated are 16 (11.04%) which were also isolated in studies of Michael et al (12.9%) and Raksha et al (4%), *Proteus* species are 12(8.2%) and *Citrobacter* 10(6.9%) which were also isolated in study of Raksha et al (4%) and (12%) respectively, *Escherichia coli* isolated in this study are 8 (5.5%) which were also isolated in Michael et al(24) study (15.49%) and *Staphylococcus aureus* are also isolated in this study 6(4.2%) which are coinciding with Najia et al study (5.6%).

Coming to the isolated organisms the formation of biofilm by them. . . which was tested using Congo Red Agar media. The biofilm formation study was also previously done by Raksha et al²⁴ which almost showed similar results. Among 30 ASB isolates 56.6% have produced biofilm on Congo Red Agar. 18(75%) out of 24 *micrococci* isolates, 13(62%) out of 21 diphtheroid isolates, 16 (88.8%) out of 18 isolates, 16(100%) out of 16 *Klebsiella* isolates, 4(33.3%) out of 12 *Proteus* isolates, 4(40%) out of 10 *Citrobacter* isolates, 5(62.5%) out 8 *Escherichia coli* isolates and 6(100%) out of 6 *Staphylococcus aureus* also produced

biofilm on Congo Red Agar.

6. Conclusion

Contact lenses are the source of eye infection in this modern era. The micro organisms can lodge in most of the contact lenses and contact lens cases. . . which in turn enter into eye due to improper sterilization of lens and lens cases. And most of the bacterial isolates obtained from these contact lenses and contact lens cases are capable of production of biofilm which was studied with the help of Congo Red Agar. Even the non-pathogenic isolates like *micrococci* and *Aerobic spore bearing bacilli* are also capable of producing biofilm. The biofilm formation is one of the virulence factor which is pathogenic enough to cause infection. Hence to avoid infections caused by contact lens users the care of both the contact lenses and lens cases is very important and sterilization should be done carefully according to the instructions given by department of ophthalmology to avoid unnecessary eye infections.

7. Conflicts of Interest

The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

8. Source of Funding

None.

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