

Study of Microbial Contamination of Contact Lens Care Solution and Contact Lens Care Practices in users of Central Gujarat.

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Abstract

Background & Method: The aim of this study is to assess the contact lens care practices of the contact lens wearers in Anand district and the contamination rate of contact lens fluid using microbial culture technique. The lens care solution samples were processed directly without enrichment by culturing on MacConkey agar, nutrient agar and blood agar, and incubated at 37°C for total 48 hours, while observing for growth at 24 hour and 48-hour incubation. The swabs were enriched in brain heart infusion broth and incubated for 24h at 37°C. Sub cultures were done on MacConkey agar and nutrient agar and were incubated at 37°C for 48 hours. The bacteria which grows were further subjected to in-vitro biofilm production assay by microtitre plate method and results were recorded. For culture examination for *Acanthamoeba* the non-nutrient agar with lawn culture of *E.coli* is inoculated directly with lens care solution observed for feeding tracks created by *Acanthamoeba* as indicator of culture positive.

Result: Total of 52 participants' samples (50.4%) found contaminated with one or more microbe out of total 103 participants participated in present study. 21 isolates (40.4%) out of these 52 were found to be biofilm producer in in-vitro biofilm production assay. Also 02 (1.94%) of the total 103 samples found positive for *Acanthamoeba* showing the feeding tracks on non-nutrient agar culture. Most lens users had a habit of changing their lens solution weekly 38(36.8%) which was significantly high in number in comparison to changing frequency of daily 1(0.9%) or monthly 13(12.6%). Only 3(2.9%) contaminated lens fluid of lens wearers had a habit of washing their hands before removing lens, which was significantly low in comparison to those who washed their hands before wearing lens 49(47.5%). Among 103 participants, 50(48.5%) contaminated lens fluid of lens wearers, did not discard their lens care solution bottle for months after opening which is significantly more in number. There are total 90(87.3%) participants, who used protective glass in possibility of exposure to dust or foreign particle.

Conclusion: In present study we conclude, Females using contact lenses more compared to males. The common user of contact lens belongs to age group of 21 year to 40 year. Students are the people who are using contact lenses commonly compared with other occupation. 52 out of 103 (50.5%) lens care solution samples were found contaminated with one or more bacteria, amongst them *E. coli* was isolated in highest numbers. Biofilm production capacity was found positive in 40.4%. Only two participants (1.9%) found with *Acanthamoeba* infection as well. Symptoms of itching and redness were present in high number in all users. Amongst them itching and discharge were more significantly present in culture positive users compared to

culture negative users. Hence it is recommended for the users to screen their lens care fluid for bacterial contamination in case if they one or both of these signs while using lens.

Keywords: contact lens, lens care practices, lens care solution, microbial contamination, *Acanthamoeba*, *Pseudomonas*, *Staphylococcus aureus*, biofilm, microtitre plate.

Introduction

Contact lenses are thin, curved plastic disks designed to cover the cornea of the eye. People use contact lens for vision correction as well as cosmetic and is placed on the cornea of the eye. The use of contact lenses has increased remarkably because of its optical, occupational and cosmetic advantages. Some of the advantages experienced by users are more attractive appearance with contact lens than with glasses, less affected by wet weather, do not steam up, and provide a wider field of vision. [1]

Microbial contamination of contact lenses during use is a major problem for contact lens user as lens care fluid is potential reservoirs of pathogenic bacteria. These bacteria have been associated with adverse corneal events such as microbial keratitis. [2] According to Anitha et al. contact lens wear accounts for approximately 12%–66% of all events of microbial keratitis. [1] The advantages and attractions of using contact lens resulted in increased use of contact lens in the last two decades, which has made users more susceptible to developing conjunctival events and serious corneal infections than non-wearers. Though these events are not well publicised but, are well-documented in scientific literature and are known to ophthalmologist. [1]

Several microbiological studies in different parts of the world have indicated consistently high rates of bacterial contamination in the contact lens cases of asymptomatic contact lens users. [3] The wearing of lenses causes changes in the cornea in terms of structure, turnover, tear production and gas exchange. [4] These changes in themselves can produce problems and may also exacerbate pre-existing conditions, which may be infectious or non-infectious in nature. [4]

Infections are commonly associated with adverse corneal events like microbial keratitis, acute red eye, peripheral ulcers, itching and blurring of vision. Gram negative bacteria, particularly *Pseudomonas aeruginosa* and *Haemophilus influenzae*, are associated with microbial keratitis and redness of eye, whereas Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* are associated with peripheral ulcers. [5]

Some infectious ocular diseases are due to bacterial biofilm formation. Biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism. Biofilms are usually found on solid substrates submerged in or exposed to an aqueous solution. [6] Given enough resources for growth, they quickly grow to become macroscopic. Biofilms are highly diverse in nature and contains many different types of microorganism that may include bacteria, protozoa, fungi and algae.

As biofilm formation begins with a transition of bacteria from the planktonic (free swimming) form to its genetically distinct sessile form. This can easily form on the surface of a contact

lens, as well as in the contact lens storage cases. This explains the persistence of organisms in contact lens storage cases increasing the risk for contact lens associated corneal infection in their users. [7]

Type of lens material used the level of hygiene and certain other handling practices by contact lens users are expected to influence the contamination of lens fluid/cases and thus the risk of infections. Published literature related to contact lens users' practices and contamination rate is scanty and available literature mentions 52% of users adhering to hand washing practices before and after lens wear and 62.8% of contamination rate in the lens fluid is reported.

Vallabh Vidhyanagar located in Anand district is an educational hub with large number of young lens users but no data is available about the practices followed by them while using lens and no data is available about the contamination rate or infection rates in contact lens users. Thus, this study was planned to find out the answers of some of these questions.

Material & Method

The study was conducted at Shree Krishna hospital, Gokal nagar, Karamsad, Anand. It is one of the biggest rural based tertiary care and teaching hospital of central Gujarat, with a capacity of 750 beds. Study was conducted after the approval of the Institutional Ethics committee (IEC) of H.M Patel Centre for Medical Care and Education, Karamsad and after obtaining the informed consent from the participants from January 2018 to March 2019. Also the authors, investigators and contributors of this study did not have any direct or indirect conflict of interest.

Source of data: People, who wear contact lens, reside in Anand district and were willing to participate in the study.

Inclusion criteria:

People who wore contact lens for more than or equal to one day within last six months.

Exclusion criteria:

People who wore contact lens but have diagnosed to have pre-existing ophthalmic disease or have undergone any ophthalmic surgical intervention.

The participant was explained the details of study and participant information sheet of present study were shared. Following that participant had been requested to sign patient information sheet and informed consent if willing to participate in the study. Participant was given questionnaire regarding contact lens practices, which took 5 -7 minutes time. Then contact lens care solution samples were collected at convenience of participant.

The lens care solution samples were processed directly without enrichment by culturing on MacConkey's agar, Nutrient agar and Sheep blood agar, then incubated at 37°C for total 48 hours, while observing for growth at 24 hour and 48-hour incubation. The swabs were enriched in brain heart infusion broth and incubated for 24 hour at 37°C. Sub cultures were done on MacConkey agar and Nutrient agar and were incubated at 37°C for 48 hours. If the bacterial growth is observed on plates, then the bacterial identification is done by manual bacterial biochemical reactions, at least up to genus level. Followed by identification the isolates were subjected to in-vitro biofilm production assay. The isolates are overnight enriched in trypticase soya broth (TSB, Himedia) and turbidity of it is adjusted to 0.5 McFarland. 100 µl of that bacterial suspension and plain TSB is mixed in flat bottom microtitre plate and incubated for 48 hours at 37°C. All isolates are tested in triplicate and positive and negative controls are also

tested with each batch. The medium or fluid is aspirated and remaining biofilms are washed with phosphate buffer (pH 6.8). Then wells are fixed with ethanol and after that stained with 1% crystal violet. The reading of absorbance is taken at 560nm in ELISA plate reader (Lisquant, Tulip). If the ratio of test isolate absorbance (average of three wells) and negative control absorbance is ≥ 2.0 then it is considered as biofilm producer and if ratio is < 2.0 then considered as biofilm non-producers. [6]

For culture examination for *Acanthamoeba*, the non-nutrient agar was used with loan culture of *E.coli*. The lens care solution was directly inoculated on loan cultured plate and then after incubation at 35-36°C for 48 hour, the plates were examined for feeding tracks of *Acanthamoeba* under low power objective of microscope. If the feeding tracks are seen then reported as positive for *Acanthamoeba*. If feeding tracks are not seen, then plates were observed for 10 days with same technique before reporting negative for *Acanthamoeba*. [11] The statistical analysis of the data was done in criteria which has significant number with finding p value (significant <0.05).

Results

In the present study, total numbers of 103 contact lens user participants were enrolled by convenient sampling. Participants were enrolled voluntarily after taking informed consent for evaluation of the contact lens care practices and detecting contamination from contact lens case fluid. For each participant, two separate left eye and right eye contact lens cases (total 206 samples from 103 participants) were tested with culture examination for bacteria and *Acanthamoeba* contamination. Total 206 samples of contact lens case fluid collected and, 104 samples (from 52 participants) were found with bacterial culture positive. We found 52 participants' both (left and right lens case) samples positive for bacterial culture. Also, in these 52 participants, there was complete matching and concordance between left and right lens case bacterial isolate for each culture positive participants. So, in this result, we have described as 52 positive samples out of 103 total samples, considering each participant's two separate left and right eye samples as one sample with similar results.

Table 1: Result of bacterial culture and Biofilm detection (total n = 103)

Culture outcome	Number of sample	In-vitro Biofilm producer numbers (n=21)	In-vitro Biofilm producer % (40.4%)
<i>E. coli</i> .	20	7	35.0%
<i>Pseudomonas aeruginosa</i>	14	7	50.0%
<i>Staphylococcus aureus</i>	7	3	42.9%
CONS (Coagulase negative <i>Staphylococcus</i>)	5	2	40.0%
<i>Klebsiella</i>	3	1	33.3%
<i>E. coli</i> , CONS	1	1	100%
CONS, <i>Streptococcus</i>	1	0	0.0%
<i>Klebsiella</i> , <i>Streptococcus</i>	1	0	0.0%
No microbial growth	51	NA	NA

Out of total 52 culture positive samples, the highest number of isolate was *E. coli* and lowest single isolate was *Klebsiella spp*. Out of these 52 isolates, total of 21 (40.4%) bacteria were found to have in-vitro biofilm production capacity. Looking to genus wise biofilm production positivity, *Pseudomonas aeruginosa* was found to have highest rate, 7 (50%) out of total 14

isolates were biofilm producer, followed by *Staphylococcus aureus* (42.9%) and Coagulase negative *Staphylococcus* (40%).

Table 2: Demographic details of study participants and culture results (n=103)

Criteria	Outcome	Culture positive (n=52)	Culture negative (n=51)
Sex distribution	Male	06	09
	Female	46	42
Age wise distribution	0 – 20 years	01	23
	21 to 40 years	51	28
Occupation	Student	34	49
	Job	05	02
	Banker	01	00
	Doctor	02	00
	House wife	06	00
	Modelling	02	00
	Teacher	02	00

Out of total 103 participants, there were total 15(14.5%) male and 88(85.5%) female, among them only 6(5.8%) male and 46(44.6%) female were having contaminated lens fluid. In age wise distribution, there were total 79(76.6%) participants belong to 21 – 40 years age. In occupation, out of total 103, it was seen that highest number of users were students 83(80.58%) and lowest was banker 01(0.97%).

Table 3: Clinical presentation and usage history of contact lens use (total n=103)

Criteria	Outcome	Culture Positive (n=52)	Culture Negative (n=51)	P value
Presence of symptoms	Itching	36	16	0.007
	Redness	34	32	
	Discharge	14	1	
	Dryness	2	1	
Eye related complains history	Conjunctivitis	3	51	1
	Stye	2	51	
	Extended wearing	3	51	
Contact lens use duration	>- 1 year	29	31	0.691
	< 1 year	23	20	
Purpose of using lens	Cosmetic	16	24	0.098
	Vision correction	34	27	
	Both	2	0	
Frequency of using lens	Regular	17	12	<0.0001
	Moderate	26	1	
	Occasional	9	38	
Lens type	Disposable	27	7	<0.0001
	Other	25	44	
Lens variety	Regular	34	27	0.184
	Coloured	17	24	
Advise taken from	Doctor	24	14	0.066
	Opticians	28	37	

(*Regular = Daily, Moderate = Once in a week, Occasional = Once in a month)

Table 3 shows correlation between culture outcome clinical presentation, lens material variety and usage history of 103 participants. Among all culture positive individuals, itching in 36(34.9%) and redness in 34(33%) were common symptoms. In case of frequency of using lens, most contaminated lens fluid were of regular 17(16.5%) and moderate 26(25.2%) lens wearers, and their primary reason for using lens were vision correction 34(33%) and cosmetic 16(15.5%) purpose. Regular lens wearers were 34(33%), contaminated more than colored 18(17.4%) contact lens wearers. (P value = 0.17).

Among 103 participants, before wearing lens 24(23.3%) contaminated lens fluid of lens wearer have consulted ophthalmologist and remaining 28(27.1%) are those who have bought them directly from optics without consulting of an ophthalmologist which are higher in number.

Table 4: Lens hygiene and eye protection practice comparing to culture outcome (total n =103)

Criteria	Outcome	Positive Culture (n=52)	Negative culture (n=51)	P value
Lens Care solution changing frequency	Daily	1	11	0.001
	Weekly	38	36	
	Monthly	13	4	
Hand washing practice	Before wearing lens	49	45	0.35
	Before removing lens	3	3	
	Both	0	3	
Using tap water	Yes	2	0	0.49
	No	50	51	
Discarding lens care solution bottle after one month of opening	Yes	02	00	0.49
	No	50	51	
Using protective glass in possibility of exposure to dust or foreign particle	Yes	41	49	0.015
	No	11	2	

Table 4 shows correlation between culture outcome and lens hygiene and eye protective practice. Most lens users had a habit of changing their lens solution weekly 38(36.8%) which was significantly high in number in comparison to changing frequency of daily 1(0.9%) or monthly 13(12.6%). Only 3(2.9%) contaminated lens fluid of lens wearers had a habit of washing their hands before removing lens, which was significantly low in comparison to those who washed their hands before wearing lens 49(47.5%). Among 103 participants, 50(48.5%) contaminated lens fluid of lens wearers, did not discard their lens care solution bottle after one month of opening it, which is significantly more in number. There are total 90(87.3%) participants, who used protective glass in possibility of exposure to dust or foreign particle.

Discussion

Many of the participants in the study (101 contact lens wearers) were young adults, below the age of 40 years and few were teenagers. This revealed that use of contact lens is very high in young adults. The microbial flora found in these contact lenses of asymptomatic wearers might be from the environment, water or from unhygienic contact lens handling practice of the wearers. *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* spp., *Pseudomonas aeruginosa*, Coagulase negative *Staphylococcus aureus* were the most common

microorganisms found in our study. The biofilm production capacity was found positive in 21 (40.4%) of total 52 isolates, which is also significant looking to prevalence, *Pseudomonas aeruginosa* was leading in genus level prevalence of biofilm producer isolates (50%), which is less compared to study published by Sherifa Mostafa et.al. [6] The occurrences of *Staphylococcus aureus* in the contact lenses used in this study is in agreement with study of M Anitha et.al [8], Sankaridurg et al. [9] and Brooks et al. [10]

On finding microbial contamination, in contact lens may or may not end up giving clinical problems in form of any eye symptoms like itching, redness, discharge or dryness on microbial colonization of conjunctiva. 33% of students in this study participated giving higher contamination and clinical symptoms. Study of Affiong Ibanga's shown that, 40.8% of students in their study enrolled to varied eye symptoms while using contact lenses. Varied eye symptoms presentation observed as itching (69.2%), redness (65.3%), discharge (26.9%), and dryness (3.8%) while using contact lenses. Redness of the eyes, foreign body sensation and itching of the eyes were commonly reported. This is similar with study of Affiong Ibanga. [13] In our study *Acanthamoeba* were detected in two female lens user's samples, but there was no significant clinical presentation noted in both the participants. Free living *Acanthamoeba* has been isolated from the dust, contact lenses, domestic water and swimming pool. Where Kamel and Norazah [11] reported the first case of *Acanthamoeba* keratitis in a female's contact lens. It has been suggested that bacteria found in eyelids, conjunctiva and tear film might have a contributory role in the pathogenesis of *Acanthamoeba* keratitis. There are further evidences that soft contact lens wearers could be at greater risk for protozoan infection due to favourable environmental conditions like presence of enough moisture in that lens user's eye. [12]

The use of contact lens is usually very popular in females, mostly for vision correction or cosmetic purpose compared with males. In assessing gender differences among the total population in present study we found that females (84.5%) used contact lens more than males (15.5%). This is like studies done in Calabar, Nigeria by Affiong Ibanga et al. [13], Owerri, South eastern - Nigeria, where Emereole et.al. [103] reported a higher percentage of females (79.3%) as contact lens wearers. Similarly, Tajunisah, Kuala Lumpur – Malaysia [14] reported majority of contact lens wearers as females. Abahussin et.al in Saudi Arabia, reported a high prevalence of contact lens users were females. This has been attributed to the cosmetic advantage offered by these lenses which tends to attract more female wearers.

Lens care is recommended by the lens care fluids only but, due to any reason sometimes people do deviate from this standard practice and may end up using tap water. Concerning contact lens care, in this study 47.5% participants washed their hands before handling contact lens, where Affiong Ibanga reported that 52% participants washed their hands before handling contact lens. [13] There were only 1.9% of participants who used tap water to clean their contact lens in this study rest all used recommended lens care solution for washing their lenses.

Conclusion

In present study we conclude, Females using contact lenses more compared to males. The common user of contact lens belongs to age group of 21 year to 40 year. Students are the people who are using contact lenses commonly compared with other occupation. 52 participants' samples (50.5%) found contaminated with one or more microbe out of total 103 participants participated in present study. Amongst them *E. coli* was isolated in highest numbers. Only two participants (1.9%) found with *Acanthamoeba* infection as well. Symptoms of itching and redness were present in high number in all users. Amongst them itching and discharge were more significantly present in culture positive users compared to culture negative users. Hence,

it is recommended for the users to change the lens care fluid on regular basis and follow standard manufacturer's instruction for safe use of contact lenses.

Vision correction was found to be purpose of using contact lens in more participants with higher contamination ratio compared to cosmetic purpose in participants. Disposable contact lens is found to be more vulnerable for microbial contamination compared to regular lens, hence with this study it is recommended to use of long-term lens should be increased compared to disposable variety.

All users may be aware about possible contamination by dangerous microbial pathogens and lens disinfectant manufacturers must develop new compounds to prevent lens care solution contamination. Also looking to bacterial tendency and capacity to form biofilm on contact lens case as well as contact lens, it is also advisable to discover novel lens and lens case material which is capable of preventing biofilm formation on them, which may improve contact lens safety and prevent contact lens associated microbial complications. We further suggest that inexpensive disposable lens storage cases be developed for use and disposal after shorter duration like two weeks. This may reduce the contaminant bacterial population and, in some cases, abolish microbial contamination. ^[13]

Further, the contamination of the lens case or lens care solution is always not ending up in clinical symptomatic ophthalmic events, as appearance of symptomatic clinical episode is also dependant on certain host factors like host immunity as well. Therefore, all users with contaminated contact lens care fluid or lens case will not develop clinical event but, anyway at risk of developing such clinical event. So, the users are advised to consult ophthalmologist in case if facing any ophthalmic event after contact lens use.

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