Biofilms Formation on Contact Lenses: Clinical and Bacteriological Study

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Abstract

Background: Biofilms can be defined as communities of microorganisms attached to a surface to prevent themselves from the environmental effects and cause infections. A study on microbial contamination associated with contact lenses wearers was carried out on women in Ramadi city, some of them were asymptomatic and others were complaining of redness, tearing and itching.

Objectives: Isolation and identification of microbial infection associated with contact lens wearers by examination of the storage case solution, studying of different factors that have effect on microbial infections among contact lenses wearers such as (age, wearing type, symptoms) and Performance of biofilm assay on the new contact lenses, Microtitre plate (MTP) and comparison with used contact lenses and the ability of biofilm formation among different isolates from storage case solution.

Material and Methods: Eighty samples of solution were collected from women of different Sources who Contact lenses: Five used and two new contact lenses as well as Microtiter plate (MTP) were used as samples to observe biofilm formation in this study. Sample from twenty new storage solution bottles were used as a control sample Isolation and identification of microorganisms: Macroscopical examination, Culture and Biochemical tests. Microscopic examination were done. According to Yukinobu, *el al.*, 2006.

Results: The study was done during the period from April 2008 to Jan. 30th 2009. Samples were collected from (80) women distributed as follows: (housewife, pupil, student, staff and others). Their ages were ranging from 13 – 36 years. The most common age of contamination was found to be ranged between 19-24 years which accumulated for (55%) of all Eye' redness is found to be the most common symptom (26.25%) (complained by the sampled women of this study. Isolates of *Pseudomonas aeruginosa, Staph. aureus, Staph. epidermidis, Liesteria and Klebsiell*a were obtained from the storage solutions of contact lenses. According to the optical density showed by Microtitre plate (MTP) Reader, Pseudomonas aeruginosa was the most common bacterial type associated with biofilm formation for the considered samples.

Conclusion: *Pseudomonas aeruginosa* was the most common bacterial type associated with Biofilm formations.

Key Words: Biofilms, Contact lenses, Infections

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Introduction

It is worldwide, approximately 100 million people use contact lenses as an alternative to spectacles (1.6%) of the world population. As the contact lens market continues to grow, public health issues associated with contact lens use have become of increasing importance. Infectious keratitis is the most devastating complication of contact lens wear and may result in permanent visual loss from corneal scarring or perforation [1].

Contact lens wear has become the most significant risk factor for corneal ulceration in patients with previous healthy eyes. With the widespread useing of contact lenses for cosmetic and optical advantages, the public health risks associated with contact lens are still an area of concern [2].

Despite advances in lens design and materials, bacterial infection and Biofilm forming bacteria a vision-threatening problem in contact lenses wearer. Insufficient knowledge of proper lens handling and use may be the important factor [3].

Materials and Methods

Collection of the Samples

Eighty samples of lenses keeping solution were collected from women (different sources), each woman was interviewed and advised answer questionnaire form was completed, the data registered included age, occupation, residential area, type of wear, sharing wear, brand, saline type, cleaning scheme, duration of use, seasonal variation and signs.

Contact lenses

Five used and two new contact lenses as well as Microtiter plate (MTP) were used as samples to observe biofilm formation in this study.

Under aseptic condition and the use of sterile forceps and blade, each contact lens was divided into 6 equal pieces and laid on a Microtitre plate (MTP). After 24 hours of incubation, the samples (CL and MTP) rinsed with phosphate buffer saline (PBS), then colored by crystal violet for 15 minutes, then rinsed again with PBS and then inserted in Microtitre plate (MTP) to see if there is a biofilm formation in contrast the optical density formed on the Microtitre plate (MTP).

Control sample

Twenty new storage solution bottles were used as a control sample. The purpose of this sample was to detect whether or not the new storage solutions are already contaminated by any type of bacteria or other microorganisms.

Isolation and identification of microorganisms

Bacteriological Investigations:

Bacteriological investigation for specimens were done on blood agar, MacConkey agar Chocolate agar aerobically and 5% Co_2 at 37 ^{0} c for 24-48 hrs. Bacterial colonies were identified using Macroscopical and Microscopical criteria and biochemical tests following [4]. Bacterial isolates were kept frozen at -20 to be used for biofilm formation experiments later.

Biofilm assay:

Small quantities of the culture were sub cultured on the BHI broth over night at 37˚c to confirm purity and viability .Most bacteria were cultivated in trypticase soy broth (TSB) supplemented with 0.25% Glucose. The ability of bacteria to form biofilms on a biotic surface was quantified essentially as following [5], bacteria was grown overnight on TSB with 0.25% glucose at 37 ^{0}C the Contact Lenses were fixed to the bottom of 96-well poltstyrene microtiter plate, Bacterial cultures were diluted 1:40 in TSB containing 0.25% glucose and 200µl of diluted culture was added to each Contact Lenses containing well for incubation at 37˚C. Each experiment was performed in five replicates wells. After incubation 24,48,72,96 hr. each CL was gently washed three times with 200µl

phosphate buffer saline (PBS) and dried. Stained with 1% crystal violet for 15 minutes. CL was rinsed again with (PBS) and bound crystal violet was solubilizedin 200µl of ethanol-aceton (80:20 vol/vol). The optical density at 630 nm was determined using a microtiter plate reader.

The same procedure was used with new Contact Lenses (unused CLs), used Contact Lenses and on microtiter plates.

Results

Out of 80 females included in this study, only 5 cases (6.25%) were found to be not contaminated, that is to say, 75 cases (93.75%) of the females were practiced contact lenses contamination. Such a percentage of contamination gives a clear idea about the prevalence of bacterial contamination among contact lenses wearers.

Age of participants

The age distribution of the females participated in this study is presented in figure 1.

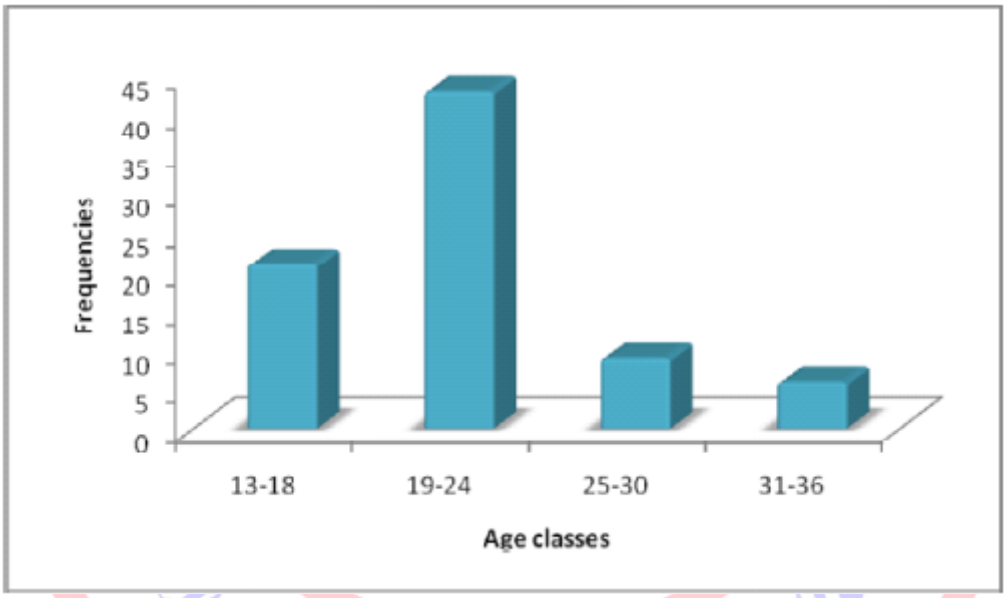

Figure (1): Frequencies of age classes of the females participated in this study.

This figure shows that most of the females (75%) fall in the age range 15-25 years, which pointed out a serious problem of vision ability at an early age of the participants, and that would be accumulated to a more serious problem if remains untreated.

Table (1): Distribution of bacterial contamination with respect to the wearing type.

Wearing Type	Frequency	Bacterial contamination			
		Postive		Negative	
		No.	%	No.	%
Daily wear	16	15	93.75	1	6.25
Occasionally wear	64	60	93.75	4	6.25

Associated symptoms

Analysis of the frequencies and percentages of the recorded signs through the questionnaire form showed that redness is the most common sign (26.25%).

Tearing and itching together accounted for the second highest percentage (20.0%).

Itching alone is accounted for the lowest percentage (7.5%).

Table 2 shows the cross classification of signs with regard to bacterial types.

Redness (32.4%) is found to be remarkably associated with bacterial type 1, whereas tearing and itching (46.7%) were mostly simultaneously occur with bacterial type (Table-2).

Table (2): Cross classification of symptoms with respect to bacterial types.

Bacteria	Symptoms							Total
	Non	Redness	Tearing	Itching	Redness and Tearing	Redness and Itching	Tearing and Itching	
Non	0	1	0	1	1	1	1	5
Pseudomonas auerogeousa	2	12	6	2	7	4	4	37
Staphylococcus epidermidis	0	0	2	2	1	0	0	5
Staphylococcus aureus	4	0	1	0	1	0	1	7
Staphylococcus spp	0	0	0	0	0	0	1	1
Haemophillus spp	0	4	1	0	0	0	1	6
Klebsiella spp	2	2	1	1	1	1	7	15
Lesteria	0	0	1	0	0	0	1	2
E-coli	0	2	0	0	0	0	0	2
Total	8	21	12	6	11	6	16	80

Bacterial contamination

Out of 80 females in this study, only 5 females (6.25%) were found to have no bacterial growth in the storage solution of their contact lenses, it is obviously that 93.75% of the sample individuals have positive bacterial growth in such solution. This result will be discussed in terms of other variables included in this study.

Types of bacteria

Table **3** shows the frequencies and percentages of bacterial growth with respect to the type of bacteria.

Table (3): Frequencies and percentages of bacterial types.

Bacteria	No.	%
Non	5	6.25
Pseudomonas auerogenousa	37	46.25
Staphylococcus epidermidis	5	6.25
Staphylococcus aureus	7	8.75
Sterptococcus spp	1	1.25
Haemophillus spp	6	7.50
Klebsiella spp	15	18.75
Lesteria	2	2.50
E-coli	2	2.50

This table indicates that ***Pseudomonas auerogenousa*** and ***Klebsiella spp***.were showing the highest percentages (46.25 and 18.75) respectively. It can be concluded that these two types were the most common types found in the storage solution of the contact lenses in this study Biofilm forming bacteria regarding to biofim formation it was found that Pseudomonas *aeruginosa* was the most common bacterial type associated with biofilm formation .as shown in Figure (5)

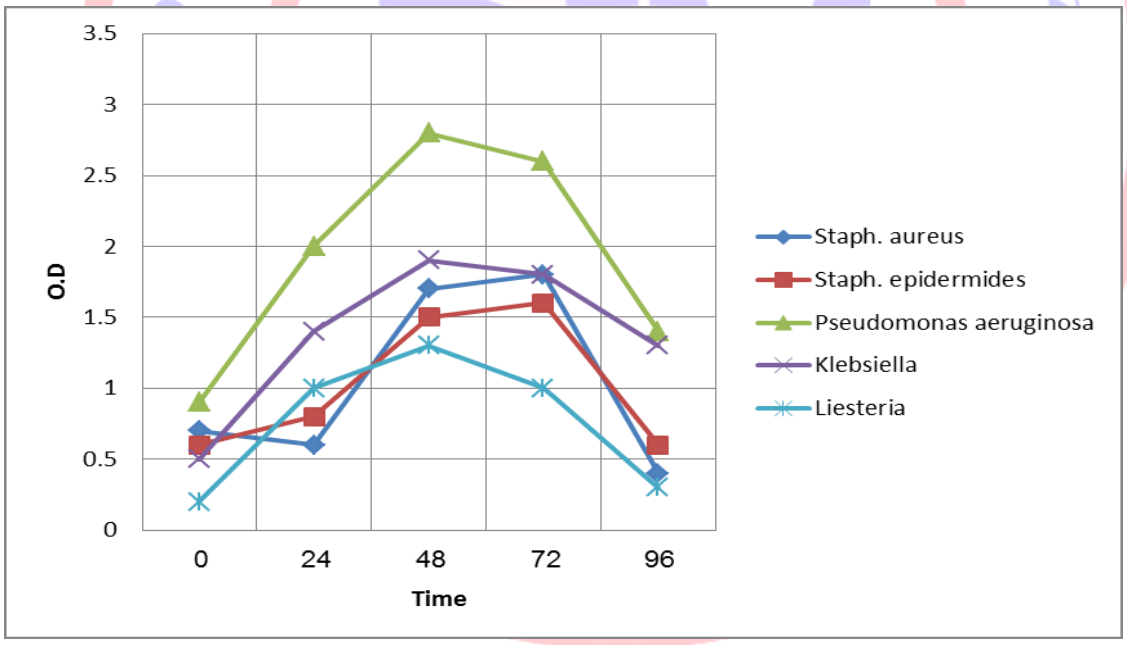

Figure (5): Average replicates (Biofilms formation) regards to Bacterial types and Time (hr.)

Discussion

It is interesting to state that type of bacteria or any other contaming microorganisms depend on the residential area of sampled individuals announced that many differences in keratitis profile have been noted between populations living in rural or in city areas, in western, or in developing countries, this statement indicates the high

Potentialities of having contaminated rural areas by which such contamination may be transferred easily to either humans or their used objects [6]. Although such a finding

contradicts is the finding of this research work, but it also may lead to the idea that urban areas are also contaminated due to hygiene ignorance for several years cultured different samples that were taken from lenses, cases and storage solutions to check for possible bacterial growth [7].

The bacteria isolated from the daily wear lens users were *Pseudomonas aeruginosa* (37.5%), *Klebsiella pneumoniae* (24%), *Escherichia coli* (16.67%), Citrobacter freundii (8.33%), *Staphylococcus epidermidis* (4.17%) and *Streptococcus pneumoniae* (4.17%). However, among the extended wear lens users the bacteria isolated were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* (4.17% each) [8].

When compared to the current research work, one can easily notice that *Pseudomonas aeruginosa* is the most type of bacterial type that associated with daily as well as occasionally wear of Contact Lenses.

It is obvious that sharing contact lenses can result in the spread of microorganisms that can severely damage the eyes, and contact lenses can spread infectious diseases. Therefore, people should only wear contact lenses prescribed specifically for them by a qualified eye care professional.

Bacterial contamination is often associated with ocular infection and inflammation during extended wear of contact lenses [9]. The coagulase-negative staphylococci, especially *Staphylococcus epidermidis* is the most commonly microbe found on contact lenses is the [10]. Accordingly there is a remarkable potentiality that contact lens surfaces varies in their ability to accumulate bacterial colonies.

The lens case was observed to be the most frequently contaminated item. Thus, the lens and its accessories need to be changed at regular intervals as users tend to become careless in lens handling inviting bacterial contamination., They also revealed that in India, most of the contact lens cleaning solutions either contain tauranol or poloxamer which when used alone might not be sufficient enough for debris removal. Supplementing the cleaning solutions with tauranol and poloxamer yielded a solution are much more effective in stimulating bacterial detachment.

Taurine an amino acid and an antioxidant protects corneal cells from osmotic stress. Of course such poor solutions will for sure contribute significantly for the process of bacterial contamination [11].

The prevalence of bacterial infections among wearers of CL was one of the aims of this study as it was for a number of other studies. The percentage of bacterial contaminated storage solutions was found to be 93.75%.

This percentage is higher than that one found by Pit, 1979, that 10 (34.5%) out of 29 storage solution cases were contaminated with bacterial infection. And it is also higher than that one found by Gray, *et al*, 1995.

Durban, *et al*., 1996, their study investigated bacterial contamination in 52 different hydrophilic contact lens solutions marketed in Spain by 12 different companies. They filtered the entire contents of 5 new, factory-sealed bottles from each of the 52 brands and cultured the filter on a neutralizing broth plate. Bacteria were cultured, isolated, and identified from 29 of the 260 bottles tested (11.15%). Eight of the 52 brands had at least 1 of the 5 bottles contaminated (15.38%). Contaminated solutions originated from four different companies.

One manufacturer contributed most of the positive cases due, presumably, to an industrial contamination by *Pseudomonas fluorescens*. The rest of the culture-positive bottles were contaminated by *Bacillus spp*.

and *Oerskovia spp*. Saline solution is also found to be used as a storage solution for CL.

In their study about bacterial contamination, Lipener et al., 1995 found that 13 subjects (86.6%) used contaminated storage solution [14].

Non-sterility of a contact lens solution may have serious health consequences, including eye infection and microbial keratitis. Three lots sold in Japan were found to have bacterial contamination, which compromised sterility.

In Hong Kong, Yung, *et al*., (2007), carried out a research work that aimed to examine the rates of microbial contamination, and identify contaminants associated with contact lenses and lens care accessories used by a group of young contact lens wearers. From the samples tested, 9% of lens extracts, 34% of case extracts and 11% of solution samples were contaminated with ocular pathogenic microorganisms. *Serratia spp*., *Staphylococcus aureus* and coagulase-negative staphylococci were the most common microorganisms isolated. Lens cases were the most frequently contaminated item. Lens cases also yielded the widest range of bacterial isolates. Contact lenses used by occasional wearers were associated with a higher contamination rate. Using either saline or multipurpose solution to rinse lenses before using was appeared to be effective in reducing incidence of contamination.

In the light of the above studies, one may realize that storage solution is a major factor in the process of Contact Lenses contamination. The extent of contamination and type of infection depends on various situations, such as the residential area, manufacturer, style of use, and many other social factors.

Gray, 1995, showed that the observation of different biofilms revealed that 77% storage cases of Contact Lenses showed bacterial growth, 24% fungi, and 20% protozoa. These results indicated why bacterial growth was the most common contaminated source of infection among Contact Lenses wearers. Accordingly this research work focused on the investigation of biofilm samples for *Pseudomonas aeruginosa* Wilson, et al., 1990, showed that biofilms adhered different types of microorganisms in both storage cases and storage solutions of Contact Lenses. Pit, et al., 2008, used biofilms of *Staphylococcus aureus* or *Pseudomonas aeruginosa* which were grown on lens storage cases and incubated with a contact lens in different multipurpose lens care solutions.

Regarding to biofilm formation it was found that *Pseudomonas aeruginosa* was the most common bacterial type associated with biofilm formation on contact lenses.

Our study agrees with the above three studies.

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