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The Relation Between Toxigenic Bacteria Contaminate Soft Contact Lenses and its Light Permeability

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Contact lenses (CL) are an innovative solution to therapeutic reasons or for cosmetic. It has several advantages compared with glasses but it has been maligned on lenses that increase bacterial contamination which may cause eye redness, inflammation, ulceration of the cornea and edema in the conjunctiva which develops for faulty vision. More than 30 pairs of soft Polymacon lenses were collected randomly from volunteers. The lenses have been divided into six groups according to the intervals used (1, 5, 10 and 15 days). The total count of associated bacteria was 4, 11, 31, 46, 71, 136 CFU per ml, respectively. The BIOLOG was used to identify bacterial isolates. The most frequent bacterial isolates were *Staphylococcus aureus, Achromobacter insolitus, Bordetella hinzii, Providencia rettgeri, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtillius* and *Enterobacter cloacae*. The ability of some bacterial isolates to excrete enterotoxins was studied through using immunological tests with TECRA for *Bacillus* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. There was a significant direct relation between period of using contact lenses and number of associated bacteria and their toxicity. The light permeability was measured at several wavelengths 210, 560 and 590 nm and there was an inverse relation between the visible light permeability (% transmission) and period of using contact lenses meanwhile, there was no any effect on ultraviolet permeability at 210 nm. This study aim to focus on injures which may occur due to use the contact lenses for long time as well as the detect the relation between bacteria contamination for lenses, toxins production and light transmittance and some eyes allergic diseases.

Keywords: Cornea, Polymacon, BIOLOG, TECRA, light permeability, contact lenses.

Contact lenses (CL) are worn for curing some refractive errors, tectonic support, prosthetic, cosmetic and therapeutic purposes. In recent years co-polymers have been incorporated into the soft hydrogel lens materials, including silicone polymers for increased oxygen permeability and phosphorylcholine to increase biocompatibility (Willcox *et al.,* 2001). For more than 20 years, many researchers have worked toward understanding why the corneas of contact lens wearers are more susceptible to infection (Evans *et al.,* 2007; Willcox, 2007; Pearlman *et al.,* 2008). Several decades of research and some major advances in lens and solution technology have not resulted in a decline in disease incidence (Fleiszig and Evans, 2010).

Contact lens wear continues to be a significant risk factor for the development of acute sight threatening corneal infections (microbial keratitis) as reported (Edwards *et al.,* 2009; Stapleton *et al.,* 2008). Devonshire *et al.* (1993) reported that the problem in contact lens wear was the presence of bacteria and other microorganisms;

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because some contact lens wearers had developed microbial keratitis. Martins *et al*. (2002) observed the presence of fungi, parasites and bacteria in contact lens swabs cultures. It has been reported that the environment, the type of contact lens (CL), the duration of wear, and the type of CL cleansing solution determined the microbial load on the contact lenses (Lee and Lim, 2003). *Staphylococcuss epidermidis*, *Staphylococcuss aureus, Enterobacter* and *Pseudomonas* species found in healthy eyes, were also observed on soft contact lenses of healthy persons (Sankaridurg *et al.,* 2000).

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen implicated in sight-threatening ocular infectious diseases such as keratitis (Green *et al*., 2008). *P. aeruginosa keratitis* is considerably more common in contact lens wearers compared with non-contact lens wearers, presumably because of the altered ocular environment. Bacterial contamination of lenses and storage cases has been reported even in association with good compliance with care and hygiene regimens. Phenotypic traits expressed in biofilms are partially responsible for the emerging resistance against. Microbial keratitis is the most serious complication associated with soft contact lens usage (Venkata, *et. al*., 2002). It involves the entry and subsequent invasion of the corneal layers by the offending pathogens, which include structural as well as enzymatic components in which are responsible for the attachment of different bacteria to the cornea. As a result of increase stress imposed by the contact lenses on the cornea, the cornea is less able to defend itself against invading bacteria. Thus, the bacterium effectively manages to overcome the eye's weakened defences, precipitating a fulminant infection (Ewbank, 1995). *P. aeruginosa* is the most frequent bacterial contaminant of contact lens care system, next are *S epidermidis*, *Staphylococcus aureus*, Coagulase negative Staph (Lee and Cabrera, 1996), Fungi, protozoa (Acanthamoeba) and viruses may also cause similar infection. A major factor that may be responsible for the development of keratitis among contact lens users is the microbial contamination of their lens care system.

The problems associated with contact lens wear are potentially sight threatening, which require rapid diagnosis and treatment to prevent vision loss. Contact lens complications include acute red eye, peripheral ulcers, infiltrative keratitis and asymptomatic keratitis (Sankaridurg *et. al*, 1999 and Borazjani *et. al.*, 2004). We conduct a study in a group of young disposable hydrogel contact lens wearers who are also a subpopulation. The lens care system includes the ophthalmic solution, lens cases and lenses used by the contact lens wearer. Furthermore, several reported cases of such ocular infections have been implicated to be due to lens care system contamination. This may be attributed to improper cleaning of the contact lenses as well as the presences of contamination in the other items of lens care system. Contamination of the lens cases or lens care solutions would most likely contaminate the contact lenses (Huang *et al*., 2001).

This investigation aim to study the potential risks of acquiring microbial keratitis among soft contact lens wearer this study was to determine the prevalence rate of bacterial colonization in hydrogel contact lenses among our study participants.

MATERIALS AND METHODS

Contact lenses collection

Thirty pair of contact lenses (CL) was collected from Saudi lady volunteer in 2016. The contact lenses which used in all trails had same material from Polymacon brand (a hydrophilic polymer of 2-hydroxyethylmethacrylate (HEMA) cross-linked with ethylene glycol dimethacrylate (62%) and water (38%).

Bacteria isolation, counting and identification

The bacteria associated with lenses isolated according to MacFaddin (2000). The collected CLs were divided to many groups according to usage periods (1, 5, 10 and 15 days). Moisturized sterile cotton swabs were using with sterile normal saline solution to isolate the bacteria from the both sides of CL, individually and then the swabs were used to make a serial dilution. One ml of dilution 1 x 10^3 was used to inoculate blood agar plates by Pour Plate technique. The inoculated

Petri dishes were incubated for 48 hours at 37°C. The replicates were 3 plates per each side of lens. The pure culture of isolated bacteria from contact lenses was identified by BIOLOG technique according to, El-Naggar *et al.*, 2012.

Determination of Bacteria toxins

The enterotoxin assay for *Bacillus* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* was carried out as recommended by the manufacturers of the kits TECRA, 3M and according to, Park *et al.*, (1992).

Light permeability measuring

The tested contact lenses (Soft Polymacon) were collected from volunteer after (0, 1, 5, 10 and 15 days) of use and directly were measured individually. Light transmission measurements were conducted using the spectrophotometer (model BECKMAN DU-640). Each contact lens was placed in a saline filled quartz cell before placing it inside the spectrophotometer. The second quartz cell was filled with saline only. The transmission spectrum was taken at 210, 560 and 590 nm. The light transmission was taken three times for each lenses and the mean and standard deviations were calculated. Each contact lens was placed inside the cell with the convex side facing the light source and the saline was replaced each time a new contact lens was tested according to, Bariah and Goh, (2005).

Statistical analysis

The data obtained were statistically analyzed using ANOVA with the MSTAT-C statistical package. The least significant difference procedure was used at the 0.05 level of probability.

RESULTS

Bacteria isolation, counting and identification

The obtained results from table referred that, 295 bacterial isolates were isolated from contact lenses through different periods of use (0, 1, 5, 10, 15 days) and under normal circumstances. All isolates belonged 8 different genus and also 8 different species. The total isolates at zero time were 2, and increased gradually with usage periods increasing 1, 5, 10, 15 days to 8, 37, 93, 155 isolates, respectively. *Staphylococcus aureus* isolates were more frequent with and its number was 45 isolates, and its frequency reached to 27.12%. *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Achromobacter insolitus*, *Providencia rettgeri*, *Bacillus subtillius*, *Escherichia coli* and *Bordetella hinzii* (32, 18, 16, 12, 11, 10, and 9, respectively,) and their frequencies were up to 21.02, 12.2, 9.49, 7.80, 7.46, 7.12 and 6.44%, respectively. Meanwhile, there were 2 unknown isolates.

Bacteria toxicity

A number of bacterial toxins were selected which can be secreted by tested bacteria and according to the most frequent and the most effective impact as *Staphylococcus aureus*, *Pseudomonas* spp. and *Bacillus* spp. toxins. Through Table 2 was found increasing of toxins production as direct proportion with increasing periods of use contact lenses from 0, 1, 5, 10, 15 days.

Table 1. Occurrence and frequency % of bacteria contaminated the contact lenses (CL) after different using periods (0, 1, 5, 10 and15 Days) on blood agar media at 33 ±2°C for 48 hours

Bacterial species	Usage periods of contact lenses (day)					Total	%
	0	1	5	10	15	occurrence	Frequency
Achromobacter insolitus	0	0	2	10	16	28	9.49
Bacillus subtillius	0	0	3	8	11	22	7.46
Bordetella hinzii	0	1	4	5	9	19	6.44
Enterobacter cloacae	1	0	6	11	18	36	12.20
Escherichia coli	1	1	2	7	10	21	7.12
Providencia rettgeri	0	0	3	8	12	23	7.80
Pseudomonas aeruginosa	0	3	9	18	32	62	21.02
Staphylococcus aureus	0	3	8	24	45	80	27.12
Uknown	0	0	0	1	1	2	0.68
Total	2	8	37	93	155	295	

The light permeability

Light permeability was measured using three wavelengths; one of them in the ultraviolet range (210 nm) and others through the range of visible light (560, 590 nm). It was found that UV permeability was almost complete throughout the tested use periods.

There wasn't any significant difference in all periods except the 1 and 5 day days. While, at 560 nm wavelength, there was a growing shortage in light permeability to 73.53% with lenses that were used for 15 consecutive days and also found a significant difference between light permeability and all using periods of lenses.

Also, in the same trend it found that reduction in the light permeability at 590 nm wavelength until reaching 70.8% only after 15 days of use the tested contact lenses. In addition there was a significant difference between all periods of use.

DISCUSSION

Contact lenses are worn for curing some refractive errors, tectonic support, prosthetic, cosmetic and therapeutic purposes. In recent years co-polymers have been incorporated into the soft hydrogel lens materials, including silicone polymers for increased oxygen permeability and phosphorylcholine to increase biocompatibility (Willcox *et al.*, 2001). For more than 20 years, many researchers have worked toward understanding why the corneas of contact lens wearers are more susceptible to infection (Evans *et al.*, 2007; Willcox, 2007; Pearlman *et al.*, 2008). Several decades of research and some major advances in lens and solution technology have not resulted in a decline in disease incidence (Fleiszig and Evans, 2010).

In the results 295 bacterial isolates were isolated from contact lenses through different periods of use (0, 1, 5, 10, 15 days) and the most isolates belonged 8 different genus and also 8

Table 2. Determination of some bacteria toxins associated with contact lenses (CL) after different using periods (0, 1, 5, 10 and15 Days) with immuno- Tecra technique

Bacterial toxin	Usage periods of contact lenses (day)					LSD
	0	1	5	10	15	at 0.05%
Bacillus spp. Enterotoxins (ng/ml)	0.01 ± 0.00	0.28 ± 0.02	0.40 ± 0.03	0.70 ± 0.06	1.20 ± 0.06	0.11
Staphylococcus aureus Enterotoxins (ng/ml)	0.00 ± 0.00	0.10 ± 0.06	1.35 ± 0.03	2.60 ± 0.06	3.90 ± 0.06	0.15
Pseudomonas spp. Enterotoxins (ng/ml)	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.05	0.10 ± 0.06	0.27 ± 0.03	0.12
LSD at 0.05%	0.01	0.12	0.14	0.20	0.18	

Table 3. Measurement of % light transmission through contact lenses (CL) after different using periods (0, 1, 5, 10 and15 Days) at different wavelengths (210, 560 and 590nm)

Usage periods of CL (days)	% light transmission		
	UV waves	Visible light	
	380 nm	560nm	590nm
0	$99.9^a \pm 0.031$	$99.5^a \pm 0.070$	$99.3^a \pm 0.030$
1	$99.7^b \pm 0.033$	$97.1^b \pm 0.088$	$96.8^b \pm 0.058$
5	$99.8^b \pm 0.033$	$89.6^c \pm 0.033$	$89.2^c \pm 0.115$
10	$99.8^a \pm 0.033$	$81.2^d \pm 0.120$	$80.6^d \pm 0.145$
15	$99.7^a \pm 0.001$	$73.5^e \pm 0.088$	$70.8^e \pm 0.058$
LSD at 0.05%	0.09	0.27	0.29

different species. The frequency of *Staphylococcus aureus* reached to 27.12% after 15 days while, *Pseudomonas aeruginosa* was 21.02% and *Enterobacter cloacae* was 12.20% meanwhile, the least frequency was 6.44 for *Bordetella hinzii* bacteria. These results matched with findings of Holden *et al.,* (1996), Sankaridurg *et. al.,* (1996) as well as Thakur and Ujjwala, (2014).

Bacillus toxins reached to 0.1, 0.28, 0.40, 0.7, and 1.2 ng/ml after 1, 5, 10, 15 days of contact lenses using and this toxins is cyclic peptide and may be occasionally cause localised eye infections in humans as mentioned by Schoeni and Wong (2005). *Staphylococcus aureus* produced a variety of toxins and reached in the trail to 0.1, 1.35, 2.6 and 3.9 ng/ml after 1, 5, 10, 15 days, respectively of contact lenses using and these toxins contribute to corneal tissue damage as recorded by Callegan *et al*., (1994). *P. aeruginosa* produced about 0.1, 0.1, 0.1 and 0.27 ng /ml of toxins after 1, 5, 10, 15 days, respectively and this results was in a harmony with recorded by Pillar and Hobden, (2002) who mentioned that, this toxins may be potentially damaging to ocular tissues and / or corneal tissue when applied exogenously to the eye

The Influence of periods of use contact lenses on its light permeability was examined during three different wavelengths 210, 560, 590 nm. It was found that, a direct correlation between time using of contact lenses and the total number of bacteria which contaminate the contact lenses. There was also, an inverse relationship between permeable lenses (% transmission) for visible light (560 and 590nm) and time of use lenses as well as total number of bacteria that contaminate the lenses. This can be explained that the length of use contact lenses increases the numbers of bacteria as a result of natural secretions of the eye and/or by external contamination. The increasing numbers of bacteria around the lenses may lead to fill the micro pores of contact lenses with bacteria cell that block light penetration. Meanwhile, There wasn't any correlation or effect with UV (210 nm) due to their ability to penetrate.

The conclusion is that, the type of contact lenses Soft Polymacon may contaminate with certain types of bacteria through eye secretions and/or external pollution. Bacteria may have a bearing on eye injury directly through the rapid reproduction and predatoct on corneal tissue or through toxins secretion that play a role in inflammation of the cornea, conjunctiva, eyelids and loss of vision. Indirectly, these toxins is consider protein which filling the micrometric pores for contact lenses that blocking light and /or reduce the permeability in addition prevent to oxygen arrival to the cornea, which consider the only origin breathing oxygen directly from the air.

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