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BACTERIAL INTERACTIONS AMONG SOFT CONTACT LENS USERS AND LENS CARE SOLUTIONS WITH ANTIBIOTIC SUSCEPTIBILITY PATTERN.

Jihan A. Mohamed [(1)], Salah M. Abdallah [(2)], Almahdy M. Alatrouny[(3)], Hatem M. Newishy [(4)].

(1)Ophthalmology Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. (2,3,4)Medical microbiology & Immunology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

ABSTRACT

PURPOSE: To evaluate the bacterial contamination associated with contact lenses and lens care solutions used by a group of soft contact lens (CL) users (daily & extended wear CL) and the susceptibility pattern of the isolated organisms to antibiotics for 12 months duration. **METHODS:** This prospective case controlled, non randomized study included 50 participants of contact lens (CL) wearers from Outpatient Clinics at Al-Azhar University Hospitals, International Eye Hospital New Damietta and available special clinics, (between 2013 and 2014). The sample population was divided into two groups: a Case group: 30 contact lens users (60 eyes) were suffering from symptoms and signs of conjunctivitis in one or both eyes; a Control group: 20 CL users were not suffering from conjunctivitis. Samples were taken from solution in contact lens storage cases; daily wear CL, extended wear CL, and conjunctiva were cultured on different media. Microbiological identification of the organisms and their antimicrobial susceptibility were done in accordance to standard protocols. **RESULTS:** In the case group, positive growth was found in 85% of the lens care solution, 65% of the contact lenses, and 56.7% of the conjunctiva of participants. While in the control group, it was found in 10% , 20% and 15% respectively, with a statistically significant difference between both groups ($P<0.001$). There was no statistically significant differences between the two usage schedules (daily wear and extended wear) regarding results of solution and contact lens cultures ($p =0.599$) and ($p=0.694$) respectively, but there was a significant higher growth in the conjunctival cultures of extended wear contact lenses ($p =0.014$). The isolated organisms in case group were; Staphylococcus epidermidis (36.3%), followed by Pseudomonas aeruginosa (34.7%), Staphylococcus aureus (15.3%), Anthracoid (5.6%), Escherichia coli (4.1%), Diphtheroid (1.6%), Non haemolytic streptreptococci (1.6 %) and least was found to be Mycoplasma (0.8%). Antibiotic susceptibility tests revealed that all isolates are sensitive to gatifloxacin, Impenem and ciprofloxacin which are commercially ophthalmological antibiotics. **CONCLUSION:** Prevention of bacterial contamination of contact lens can reduce the risk of developing ocular infections. Lens care practices amongst the participants were not optimum which resulted into high contamination level. Hence, creating awareness among the users about the lens care practices and regular cleaning and replacements of lens cases are required.

Keywords: Contact lens infections, lens care accessories, microbial contamination, Antibacterial susceptibility.

Introduction:

CLs are a safe and effective vision correction and the wearers have many choices as continuous wear, frequent replacement or daily disposable lenses [(1)].

Bacterial keratitis is a sight-threatening contact lens complication. Wearing contact lens is the main risk factor, and sleeping with contact lenses is the major risk factor among contact lens wearers [(2)].

CL can act as a vector for microorganisms to adhere to and transfer to the ocular surface. Commensal microorganisms that uneventfully cohabitate on lid margins and conjunctiva and potential pathogens that are found transiently on the ocular surface can inoculate CL in vivo. In the presence of reduced tissue resistance, these resident microorganisms or transient pathogens can invade and colonize the cornea or conjunctiva to produce infection [(3)].

The corneal surface may breakdown forming a small corneal abrasion, due to routine lens use. Presence of CL as a foreign body in the eye leads to dry eyes. So the microbial keratitis has become an increasingly important problem in recent years [(4)]. The microbial contamination of CL care product is a major problem for contact lens wearers. Other factors related with CL uses, such as duration of use, frequency of cleaning and change of contact lens lead to microbial contamination [(5)]. CL related keratitis is a serious impediment of contact lens wears, with nearly one out of five hospitalized cases needing corneal transplantation[(6)]. The incidence of contact lens related microbial keratitis has been enhanced in developing countries [(7)].Infectious conjunctivitis is mainly bacterial (approximately 78% to 80% of cases being bacterial in origin) [(8)].

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The ideal method of treating bacterial conjunctivitis is to identify the causative organism and initiate specific antimicrobial treatment known to be effective against it. Most commonly staphylococcus species in adults, and Streptococcus pneumonia and the Gram-negative organisms Haemophilus influenza and Moraxella catarrhalis in children. Contact lens users are at particular risk for Gram-negative infections. Such as Pseudomonas aeruginosa. Neisseria gonorrhoeae is primarily a neonatal etiology [(9)]. Epling [(10)] reported that the causative agents of bacterial conjunctivitis and keratitis in contact lens users are more frequently gram-negative bacteria (such as Pseudomonas aeruginosa), but may include all of the above agents.

Subjects and Methods: The sample population involved in this study was 50 contact lens users from Outpatient Clinics at Al-Azhar University Hospitals, International Eye Hospital New Damietta and available special clinics (between 2013 and 2014). The sample population was divided into two groups: a Case group: 30 contact lens users (60 eyes) were suffering from symptoms and signs of conjunctivitis in one or both eyes; a Control group: 20 contact lens users were not suffering from conjunctivitis. The study was approved by the Ethics Board of Al Azhar University and an informed written consent was taken from each participant in the study. The participants were instructed to write a complete questionnaire, which consisted of systematic questions regarding name, age, sex, systemic diseases, systemic medications, eye medications, History of redness, itching and type of discharge were recorded if present, type of lens, wearing schedule as well as disinfection schedule. The age of patients ranged from 18 to 53 years old they included 3 males & 47 females. All participants wear soft contact lens users, either disposable extended wear lenses or conventional daily soft contact lenses. All selected individuals were free from any systemic diseases during investigation such as diabetes mellitus, liver disease or kidney disease. They did not receive any antibiotic medication (systemic nor ocular) during the study. All individuals were conducted to do blood sugar, complete blood count, ESR, liver and kidney function tests, and they had normal values.

The sample population was subjected to the following:-

(A) Clinical examination: using slitlamp biomicroscopy through Diffuse illumination, Direct (focal) illumination, narrow beam (optic section), scleral scatter, Indirect illumination and Retro illumination.

- To determine position, depth and size of corneal affection. And to evaluate the cornea and tear film with fluorescein stain, tear breakup time and corneal integrity.

- Looking for signs of secondary infection, epithelial defects, corneal cellular infiltrate, anterior chamber reaction, conjunctival injection. and the presence of discharge, lid edema.

- Everting the upper eyelids of both eyes and inspecting the superior tarsal conjunctiva for papillae.

- Inspect contact lenses for the presence of deposits, sharp edges, and cracks.

(B) Bacteriological examination: Swabs were obtained from (1) contact lens, (2) from available contact lens storage materials, and from (3) Palpepbral conjunctiva of contact lens users. CLs were removed aseptically after hand hygeine (using plain soap &water followed with alcohol rub) and wearing clean gloves then swabbed by using separate sterile cotton swabs moistured with sterile normal saline solution for each contact lens (right & left contact lens). Solution samples were taken by sterile cotton swabs. Palpebral conjunctiva was gently rubbed by separate sterile cotton swabs (keeping the eye lids wide apart to avoid contamination from lid margins). Each swab obtained was put immediately in a separate sterile tube which contains 3ml of sterile brain heart infusion (BHI) broth as a transport media and transported to the loboratory.The BHI broth containing swab were incubated at 37°C for 24 hours. Then each tube was gently streaked on 2 blood agar, chocolate agar and MacConkey agar for separate colonies. One of blood agar and MacConkey agar plates were incubated aerobially at 37°C for 24-48 h .The other blood agar plate was incubated anaerobically using gas generating system kit anaerobic system(oxoid) at 37°C for 48h. The chocolate agar plate was incubated in a CO2-enriched atmosphere (5-10%) using gas generating system kit carbon dioxide system(oxoid) &anearobic jar at 37°C for 48 h. Swabs obtained for Mycoplasma were inoculated directly on Mycoplasma agar media(Difco) and incubated aerobically for up to 7 days with 5–10%

CO_2 at 37°C. Bacterial isolates were identified by standard microbiological techniques : Gram staining, character of the colonies,biochemical tests, including ctalase, coagulase, hemolytic activity on blood agr plaes for Gram +ve organisms, and sugar fertementation, indole, citrate utilization, urease, oxidase, triple sugar iron, and voges proskauer tests for Gram-ve bacilli.

Antibiotic susceptibility testing: The antibiogram was done for isolated strains by disk diffusion method against Amoxicillin 25, Amoxicillin/ Clavulinic acid 30, Erythromycin 15, Tetracycline 30, Cefotaxime 30, Cefoxitin 30, Tobramycin 10, Gentamicin 10, Ceftriaxone 30, Ciprofloxacin 5, Gatfloxacin 5, Ofloxacin 5, Imipenem 10, Chloramphenicol 15, and Vancomycin 30.

Reading and interpretation: the plates were examined after 24 hours incubation, and the diameter of the zones of complete inhibition was measured. The zone diameter for individual antibiotic was translated into sensitive, intermediate, and resistant by referring to an interpretative table according to the Clinical Laboratory Standards Institute (CLSI) **[11].**

Statistical Analysis: Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21. Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between the 2 groups with respect to normally distributed numeric variables were done using the t-test. For categorical variables, differences were analyzed with $\chi 2$(chi square) tests and Fisher's exact test when appropriate. All p-values were two-sided. P-values < 0.05 were considered significant

Results: The case group included 2 males (6.7%) and 28 females (93.3%) and a mean age 25.8 ±6.5. While control group included, 1 male (5.0%) and 19 females (95.0%) with a mean age 24.3 ±3.7. There was no statistically significant differences between the two groups (p=0.375) (p=0.808) respectively as regard age and sex. As regards bacterial growth in the case group, positive growth was found in 51(85%) of the CLs solution, 39 (65%) of the CLs, and 34(56.7%) conjunctiva of the participants. While in the control group, it was found in 4(10%), 8(20%) and 6(15%) respectively, with a significant statistical difference between both groups (P<0.001)(Table 1).

Table (1): Comparison between case group and control group regarding results of microbial growth.

Factors	No of eyes		Test value	P value	Significance
	Cases n=60(%)	Controls n=40(%)			
Case Solution					
No growth	**9(15.0)**	**36(90.0)**	**χ^2 =54.54**	**<0.001**	**significant**
Growth	**51(85.0)**	**4(10.0)**			
Contact lens					
No growth	**21(35.0)**	**32(80.0)**	**χ^2 =19.51**	**<0.001**	
Growth	**39(65.0)**	**8(20.0)**			**significant**
conjunctiva					
No growth	**26(43.3)**	**34(85.0)**	**χ^2 =17.36**	**<0.001**	**significant**
Growth	**34(56.7)**	**6(15.0)**			

There was statistically significant differences between the two study groups (case group and control group) regarding results of different microbial growth ($p<0.001$), ($p<0.001$) and ($p<0.001$) respectively.

Bacteria isolated from solution in contact lens storage cases:

Table 2: Organisms isolated from solution in contact lens storage cases (Total 51)

Organisms	Number	%
P. aeruginosa	22	43.1
S. epidermidis	14	27.5
S. aureus	7	13.7
E. coli	5	9.8
Anthracoid	3	5.9
Total	51	100

P, pseudomonas; S, staphylococcus& E, Escherichia

This table reveals that the organisms isolated from solution in contact lens storage cases were; 22 *Pseudomonas aeruginosa* with percentage 43.1%,14 *Staphylococcus epidermidis* with percentage 27.5%, 7 *Staphylococcus aureus* with percentage13.7%, 5 Escherichia coli with percentage 9.8% and 3 *Anthracoid* with percentage 5.9%.

Bacteria isolated from contact lens:

Table 3: Organisms isolated from contact lens (Total 39)

Organisms	Number	%
S. epidermidis	17	43.6
P. aeruginosa	12	30.8
S. aureus	7	17.9
Anthracoid	2	5.1
Diphtheroid	1	2.6
Total	39	100

P, pseudomonas& S, staphylococcus

This table means that the organisms isolated from contact lens were; 17 *Staphylococcus epidermidis* with percentage 43.6%, 12 *Pseudomonas aeruginosa* with percentage 30.8%, 7 *Staphylococcus aureus* with percentage 17.9%, 2 *Anthracoid* with percentage 5.1% and 1 *Diphtheroid* with percentage 2.6%

Table (4): Comparison between daily wear contact lenses and extended wear contact lenses regarding results of different items

Factors	No of infected eyes		Test value	P value	significance
	Daily usage n=38(%)	Extended usage n=22 (%)			
Solution					
No growth	**5(13.2)**	**4(18.2)**	χ^2 **=0.276**	**0.599**	**Not significant**
Growth	**33(86.8)**	**18(81.8)**			
Contact lens					
No growth	**14(36.8)**	**7(31.8)**	χ^2 **=0.155**	**0.694**	**Not significant**
Growth	**24(63.2)**	**15(68.2)**			
Conjunctiva					
No growth	**21(55.3)**	**5(22.7)**	χ^2 **=6.004**	**0.014**	**Significant**
Growth	**17(44.7)**	**17(77.3)**			

There was no significant statistical differences between the two usage schedules (daily wear and extended wear) regarding results of solution and contact lens cultures (p =0.599) and (p=0.694) respectively but there was a significant difference regarding results of conjunctival cultures (p =0.014).

Table 5: Organisms isolated from conjunctiva (Total 34)

Organisms	Number	%
S. epidermidis	14	41.2
P. aeruginosa	9	26.5
S. aureus	5	14.7
Anthracoid	2	5.8
Non haemolytic strept	2	5.8
Diphtheroid	1	3
Mycoplasma	1	3
Total	34	100

S, staphylococcus; P, pseudomonas; Strept, streptococcus

This table shows that the organisms isolated from conjunctivae were;14 *Staphylococcus epidermidis* with percentage 41.2%, 9 *Pseudomonas aeruginosa* with percentage 26.5%, 5 *Staphylococcus aureus* with percentage 14.7% and 2 *Anthracoid* with percentage 5.8%, 2 *Non haemolytic strepteptococci* with percentage 5.8%, 1 *Diphtheroid* with percentage 3% and 1 *Mycoplasma* with percentage 3%.

Different isolated organisms included in case group:

Table 6: Frequency of isolation of different organisms included in case group.

Isolated organism	**Number**	**%**
S. epidermidis	**45**	**36.3**
P. aeruginosa	**43**	**34.7**
S. aureus	**19**	**15.3**
Anthracoid	**7**	**5.6**
E. coli	**5**	**4.1**
Diphtheroid	**2**	**1.6**
Non haemolytic strept	**2**	**1.6**
Mycoplasma	**1**	**0.8**
Total	**124**	**100**

S,staphylococcus; P, pseudomonas; E, Escherichia; Strept, streptococcus

The isolated organisms in case group were; Staphylococcus epidermidis (36.3%), followed by Pseudomonas aeruginosa (34.7%), Staphylococcus aureus (15.3%), Anthracoid (5.6%), Escherichia coli (4.1%), Diphtheroid (1.6%), Non haemolytic streptreptococci (1.6 %) and least was found to be Mycoplasma (0.8%)(Table2) .

Table (7): Antibacterial susceptibility results of isolated organisms:

Antibiotic	Isolated organisms						
	S. Epidermidis	S. Aureus	Anthracoid	Non hem.strpt	Diphtheroid	P. aerugenosa	E. Coli
Amoxicillin	R	R	R	S	I	R	R
Amoxicillin/ Clavulinic acid	I	I	S	S	S	R	I
Erythromycin	S	S	I	S	S	R	R
Vancomycin	S	S	S	S	S	R	R
Chloramphenicol	I	I	S	S	I	R	I
Tobramycin	R	R	I	R	R	S	S
Gentamycin	I	I	S	R	R	S	S
Tetracycline	S	S	S	S	I	I	R
Cefotaxime	R	R	S	R	R	I	S
Ceftriaxone	R	I	R	S	R	I	S
Cefoxitin	I	S	R	I	I	R	I
Gatfloxacin	S	S	S	S	S	S	S
Ciprofloxacin	S	S	S	I	I	S	S
Ofloxacin	S	S	S	I	I	I	S
Impenem	S	S	S	S	I	S	S

S, staphylococcus; P, pseudomonas; Non hem.Strept, non hemolytic streptococcus and E, Escherichia S, sensitive; I, intermediate and R, resistant.

Discussion

CL is a corrective, cosmetic or therapeutic lens usually placed on the cornea of the eye; less affected by wet weather, do not steam up, and provides a wider field of vision. CL wearers are more likely susceptible to higher rate of conjunctival and corneal infections than non–wearers. Infectious keratitis is the most devastating complication of contact lens wearer and may result in permanent visual loss from corneal scarring or perforation [(1)].

For a favorable outcome, it is essential to identify the causative agent. It is known that microorganisms can reside on lenses and lens storage cases. CL solutions also act as reservoirs for microbial growth [(12)].

Bacteria are adherent to the contact lens rather than colonising the ocular surface or eyelids, which is consistent with the observation that symptoms rapidly subside once the lens is removed [13].

In the current study; there was no statistically significant differences between the two groups (p=0.375) (p=0.808) respectively as regard age and sex.

In the current study; There is a slightly higher (P=0.694) incidence of contamination of extended wear over daily wear, And this in agreement with **Rahim *et al.*** [14] study ; they reported that among the 65% contaminated lenses, more than half were extended wear, while the rest were daily wear. The is significantly higher (P<0.05) bacterial growth in the patients conjunctiva of extended wear over daily wear ,this may be due to their higher water content which are likely to pick up debris, including microorganisms which have the potential to cause eye infections. This in contrast with the study of **Hesam *et al.*** [15] who reported that 42.3% were extended wear contact lens users, while 57.7% were daily lens wearers.

CLs can interfere with typical epithelial proliferation and differentiation that may compromise barrier function. Lenses impact on innate defenses (and microbial virulence) are more probable to be prevalent with extended wear or overnight recognized risk factors of infection, while daily wear is similarly related to microbial keratitis.

In addition size contamination of lenses, the chronic hypoxic stress due to prolonged contact between the lens and the eye of the user can compromise the epithelial barrier against the infections. Such condition serves as an invitation to the potential pathogenic microorganism [16].

The most common bacteria isolated from contact lenses are coagulase-negative Staphylococci and this results agreed by the work done by **Gopinathan *et al.*** [17]. In the current study; S. aureus was isolated from 13.7% of contact lens storage case, 17.9% of contact lenses and 16.7% of conjunctiva, but **Rahim *et al.*** [14] were reported that S. aureus was isolated from 5.6% of contact lens care systems, 12.3% of contact lenses and 9.8% of conjunctiva.

Benhmidoune *et al.* [18] reported that 47.8% of their studied subjects had positive corneal bacterial cultures. The most common Pathogens recognized were Staphylococcus aureus and Pseudomonas aeruginosa. While in our study, the predominant organism is S. epidermidis followed by Pseudomonas aeruginosa. Which may be due to the high incidence of staphylococcal carriers in our country.

On the other hand, **Hesam *et al.*** [15] reported that Pseudomonas aeruginosa were the main causative agents of contact lens associated microbial keratitis, accounting for (80%) in positive cultures followed by Staphylococcus aureus 12%.
In the present study S. epidermidis was found to be the most frequent contaminant (10%) in aymptomatic subjects, which are also the most common microorganism in the normal conjunctival flora (7.5%) due to their virulence factors.

In the current study; Anthracoid was isolated from 5.9% solution of storage cases, 5.1% of contact lenses and 5.8% of the participants conjunctiva. While in a study of **Rahim *et al.*** [14], anthracoid was isolated from 10.1% solution of storage cases, 7.7% of contact lenses and 6.3% of conjunctiva. As the bacillus spores survived multiple lens disinfection treatments. Above results suggest that contact lens chemical disinfection systems should be capable of killing Bacillus species.

The use of tap water and lack of air-drying of lens cases contaminate not only the cases but also the lenses, which are stored in them. Thus, it has been suggested that lens cases must be washed with soap and clean water, disinfectant solution, wiped with clean tissue paper and then air-dry keeping away from dust.

The results of antibiotic susceptibility pattern of isolated organisms in Table 5 indicate that all of the microorganism cases were sensitive to gatifloxacin, Impenem and ciprofloxacin which are commercially ophthalmological antibiotic used. While mycoplama was highly sensitive to tetracycline, erythromycin and chloramphenicol. Which are commercially ophthalmologically available.

Conclusion:

This study revealed that there is slightly higher incidence of contamination of extended wear over daily wear due to their higher water content which is likely to pick up debris, including microorganisms which have the potential to cause eye infections. And revealed importance of referring all contact lens wearers with suspected corneal infection to ophthalmologists for culture from conjunctiva, CL and solution to guide antibiotic therapy.

Recommendation: Contact lens practitioners should inform contact lens users about the risk of microbial conjunctivitis, the need for patient compliance, and prompt assessment of contact lens-related complaints. Future study should be performed on a large scale of population for more accurate evaluation.

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