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Microbiological Contamination Linked to the Usage of Mascara among Waterproof and Non-Waterproof Brands and Tear Film Assessment

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ABSTRACT

Introduction and Aim: Microorganisms are normally found on human eyelashes. Applying mascara on lashes has the potential to infect the mascara tube with microorganisms. Constant use of eye cosmetics may also affect tear film stability, resulting in decreased tear production. This pilot study in a real-world context looked at the microbiological contamination of two mascara brands worn daily for two months, as well as tear assessment before and after application. **Methods and Materials:** Sixty women aged 18 to 39 were randomly allocated to apply one of two brands of waterproof mascara or a non-waterproof brand on both upper and lower eyelids every day for two months. Microbial contamination for various Gram-positive and Gram-negative organisms was detected using the streak technique of culture and Gram staining after two months of mascara use. Tears were assessed before and after the first and second months of mascara usage, followed by a post-study questionnaire. **Results:** There was no growth of bacteria amongst waterproof mascara users. In non-waterproof users, *Staphylococcus aureus* growth was found in 33.3% of users, *Micrococcus* growth among 23.3% and both among 15.0%. Tear assessment showed a statistically significant difference between before and after the first month of mascara use and a highly statistically significant difference between before and after the second month of mascara use. **Conclusion:** Both waterproof and non-waterproof mascara are not safe for constant use.

Keywords: Bacteria; Cosmetics; Mascara; Microbial Growth; Tear Film

INTRODUCTION

Bacteria that cause serious infections often are organisms native to the human body[1,2]. Facial skin is covered with bacteria that are part of the normal ocular flora. Most require oxygen to survive[3]. *Haemophilus* species, *Staphylococcus* sp., *Pneumococcus* sp., *Corynebacterium* sp., and *Streptococcus* sp. bacteria are commonly found on, or near, the eye. Although usually not harmful, under the right circumstances these bacteria frequently cause dangerous ocular infections[1,2]. A scratch or an abrasion in the corneal epithelium can allow these microorganisms to gain entry to the cornea and cause infection. Some pathogenic species can penetrate an intact corneal epithelium. *Neisseria gonorrhoeae, Haemophilus aegyptius, Corynebacterium diphtheroid*, and *Listeria* sp. can penetrate an intact epithelium[4]. When mascara is used at the same time contact lenses are worn, or used at constant intervals without replacement the bacterial flora around the ocular surface may be increased. which may invade into the mascara tube or any other eye cosmetics. Therefore, the control of bacterial growth in the mascara tube is important to minimize the risk of infection[5].

The skin contains 10^2 to 10^4 organisms per centimetre square. Bathing has little effect on the resident flora of the skin. Low pH, fatty acid in sebaceous secretions and the presence of lysozyme are important factors for eliminating nonresident microorganisms from the skin. Other microorganisms include *Peptococcus, Enterococcus, Micrococcus, E. coli, Streptococcus*, and *Morexella*, Penicillin resistant *Staphylococci* have also been found in skin flora.

Up to a certain extent these do not cause any severe infections because they have a beneficial role and disease production. Different bacteria are present as normal flora on different sites. i.e., skin, nose, mouth, upper respiratory tract, genitourinary tract, gastrointestinal tract, etc., The conjunctiva is relatively free from the bacteria due to the flushing action of tear and the presence of lysozyme in it. The predominant organisms are *Corynebacterium xerosis, Staphylococcus epidermidis, Moraxella species* and non-hemolytic *Streptococci*. Bacteria that mainly cause eye infection include *Pneumococci, Staphylococcus aureus, Moraxella lacunata, Chlamydiae, Neisseria gonorrheae, Pseudomonas* etc. Constant use of eye cosmetics may also influence tear film stability[5], which may decrease tear production. Eye cosmetics are obviously applied near the ocular surface. While eye shadow and mascara are applied to the peri-ocular skin and eyelashes, respectively, eyeliner is often applied millimetres away from the lid margins or directly on the lid margin[6].

During routine slit-lamp examination, particles of cosmetic products are often seen suspended in the tear film. Poor manual dexterity, application technique or eye rubbing may be one route of entry for these products to enter the tear film; however, substances applied to periocular skin demonstrate slow and eventual migration into the tear film, which can disrupt tear film stability and reduce ocular comfort[7] and may cause some contamination of microbes and fungi because of contamination of eye cosmetics. The use of cosmetics for a longer time or contamination associated with the cosmetic itself by sharing with many leads to dangerous ocular infections.

Manufacturers use preservatives to keep cosmetics free from microbial contaminants. Among which two main preservatives which can cause the heavy growth of *Staphylococcus epidermidis* and *Psuedomonas aeruginosa* are often imidazolidinyl urea or p-hydroxybenzoate than others like p-hydroxybenzoate[8]. However, the repeated use of mascara by multiple users at cosmetic counters creates greater exposure to contaminants. Repeated use of a mascara tube by a single user gives the same effect but at a slower rate[8]. The FDA established the Cosmetic Ingredient Review Program in 1976 to monitor the ingredients used in cosmetics. This program tests thousands of ingredients. Before a new product can be put on the market for consumer use, an expert panel must evaluate the item so that ingredients are ensured to be safe and efficacious. Ingredients are categorized as either 'safe as used, safe with qualifications, unsafe, or insufficient evidence'[9]. The panel then puts a label on each product with the appropriate indications, warnings, and active ingredients[10]. Once a product is manufactured, contamination must still be monitored. Contamination may be easy to determine, especially if a product has changed in color, odor, clarity, or thickness[11,12]. 'Total Aerobic Microbial Count (FN1)' is the standard test used in the cosmetic industry to determine the density of microorganisms[11].

Self-preserved cosmetics typically contain ethanol, propylene glycol, or glycerol[10]. However, many manufacturers use ingredients such as tetrasodium edetate and trisodium as preservatives. Antimicrobials such as parabens and phenoxyethanol may also be used as part of the formulation of cosmetic products[13–15]. To determine microbial content, we used culturing techniques to isolate individual microbial types. Culturing can yield undesirable results unless steps are taken to maximize recovery[16]. This study looked at microbial contamination of 2 brands and the tear assessment. Waterproof brand and non-waterproof brand of two mascaras investigated were 2 of the top 10 cosmetics used among teenagers in and around Mangalore, Karnataka[17].

MATERIALS AND METHODS

This study was conducted in 60 females between the ages of 18 and 39 from AJ Institute of Allied Health Sciences, Mangalore, Karnataka. Ethical clearance was obtained before commencing the study. Potential subjects completed a pre-study questionnaire. If a current eyelid disease, an eyelid disease from the past 6 months, or topical or oral antibiotic use was noted on the pre-study questionnaire. Subjects allergic towards any eye cosmetics, burning or itching of eyes, a mucous or watery discharge from the ocular surface, eyelid pain, irritation or trauma were noted and excluded from the study. Women over 40 years old were also excluded from the study because dry eye problems are more prevalent in women as they age. Individuals with contact lenses were also excluded due to the risk of high contamination. Subjects completed a pre-study questionnaire to rule out the exclusion criteria. Informed consent was obtained from 60 women who agreed to participate in the study. No specific hygiene regimen was given. The women were assigned randomly to use 1 of 2 brands of either non-waterproof or waterproof mascara which is unused, unexpired mascara purchased by the examiner based on the most frequently used brands among teenagers in Mangalore (Karnataka) population, which was then distributed to each subject at the beginning of the study (Figure 1). Subjects were asked to use the mascara daily, on the upper and lower lashes of both eyes, for 2 months.

To track compliance with mascara usage, subjects were given a log sheet to record the days the mascara was worn. Subjects were allowed to use other cosmetics but were asked not to wear anything on the eyelashes besides the assigned mascara. At the end of 2 months, subjects were available for an objective examination and sample collection. Each available subject had a brief ocular examination and was asked to complete a post-study questionnaire after 2 months of mascara use. TBUT and shimmer tests (1 and 2) were carried out on all the subjects before and after mascara use. After collecting all the samples, the culture swabs from each sample were plated on blood and chocolate agar media plates. The plates were equilibrated to room temperature at the time of culturing and inoculated using the streak method. Immediately after culturing, all plates were placed in an incubator for 32 hours at 37°C and then evaluated.

Each plate was examined for microbial growth characteristics and number of colonies. Microbes were identified using the Gram staining method. Gram-positive as well as Gram-negative were determined and further confirmed by confirmatory tests (Figures 2 and 3). After the completion of the study, post-study questionnaires were also evaluated.

Fig. 1: Sample of mascara tubes assigned (waterproof and non-waterproof)

Fig. 2: Confirmatory test: Selenite F broth

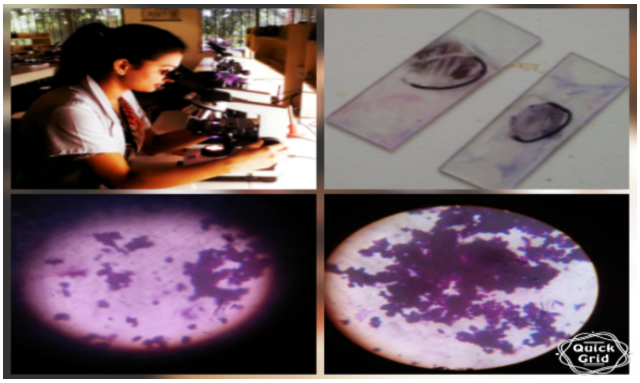

Fig. 3: Confirmatory test: catalase, coagulation test

RESULTS

None of the study indicated any symptoms of itching, burning, irritation on pre-study questionnaire. A total of 60 female subjects were enrolled in this study, 30 of them were assigned waterproof mascara and 30 were assigned non waterproof mascara. Mean age of subjects was 19.63±1.04 (waterproof users) and 19.65 ±1.07 (non-waterproof users). The result of this study showed that there is no growth of bacteria amongst waterproof users.

Non waterproof users, among 4 mascara tubes (13.3%) there was no growth, *Staphylococcus* aureus growth among 10 mascara tubes (33.3%), *Micrococcus* growth among 7 mascara tubes (23.3%) and both *Staphylococcus* and *Micrococcus* amongst 9 mascara tubes (15.0%) shown in Table 1.

Table 2 shows the statistically significant difference between before mascara use and after the first month of mascara use in Shimmers test 2 and a highly statistically significant difference before and after the second month of mascara use.

Table 1: The growth of microorganisms in waterproof and non-waterproof users

Variable (Growth)	Waterproof users	Non waterproof users	Total
No growth	30 (100%)	4 (13.3%)	34 (56.7%)
Both	0 (0%)	9 (30.0%)	9 (15.0%)
Micrococcus	0 (0%)	7 (23.3%)	7 (11.7%)
Staphylococcus aureus	0 (0%)	10 (33.3%)	10 (16.7%)
Total	30 (100%)	30 (100%)	60 (100%)

Table 3 shows that there is a highly statistically significant difference between before mascara use after 1 month and thesecond month of mascara use.

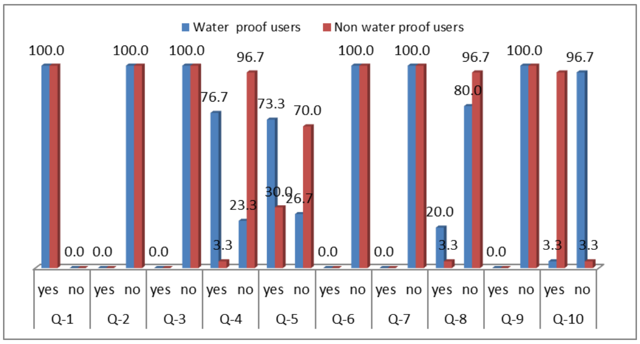

Fig. 4: Post-study questionnaire among waterproof and non-waterproof users

Figure 4 shows that, the Q-1 (100%-yes) states that the mascara was used regularly during a given time in both waterproof and non-waterproof users. Q-2 (100%-no) states that the subjects have not undergone any treatment for

Table 2: Shimmers test 1 and 2 among waterproof and non-waterproof users

Waterproof users			Mean	Mean difference	Standard error	P value	
OD	Before mascara use S1	First month S1	31.13	-0.400	0.149	0.175	NS
		Second month S1	26.63	-0.600	0.238	0.261	NS
	Before mascara use S2	First month S2	31.53	0.667	0.194	0.027	Sig
		Second month S2	25.97	1.633	0.256	0.000	HS
	First month S1	Second month S1	31.73	-0.200	0.182	1.000	NS
	First month S2	Second month S2	25.00	0.967	0.265	0.015	Sig
OS	Before mascara use S1	First month S1	31.60	-0.300	0.240	1.000	NS
		Second month S1	26.30	-0.767	0.400	0.980	NS
	Before mascara use S2	First month S2	31.90	0.600	0.228	0.202	NS
		Second month S2	25.70	1.633	0.256	0.000	HS
	First month S1	Second month S1	32.37	-0.467	0.218	0.617	NS
	First month S2	Second month S2	24.67	1.033	0.357	0.107	NS
Non-waterproof users			**Mean**	**Mean difference**	**Std. error**	**P value**	
OD	Before mascara use S1	First month S1	33.47	-0.133	0.213	1.000	NS
		Second month S1	28.30	-0.267	0.283	1.000	NS
	Before mascara use S2	First month S2	33.60	0.733	0.151	0.001	HS
		Second month S2	27.57	1.867	0.383	0.001	HS
	First month S1	Second month S1	33.73	-0.133	0.178	1.000	NS
	First month S2	Second month S2	26.43	1.133	0.324	0.023	Sig
OS	Before mascara use S1	First month S1	33.37	-0.100	0.182	1.000	NS
		Second month S1	28.63	-0.100	0.297	1.000	NS
	Before mascara use S2	First month S2	33.47	1.233	0.218	0.000	HS
		Second month S2	27.40	2.500	0.454	0.000	HS
	First month S1	Second month S1	33.47	0.000	0.235	1.000	NS
	First month S2	Second month S2	26.13	1.267	0.404	0.058	NS

S1-Shimmers 1; S2-Shimmers 2. OD = Oculus dexter [right eye]; OS = Oculus sinister [left eye]

Table 3: TBUT among waterproof and non-waterproof users

Waterproof users							
TBUT [seconds]			**Mean**	**Mean Difference**	**Std. Error**	**P value**	
OD	Before mascara use	First month	10.50	0.600	0.132	.000	HS
	Before mascara use	Second month	9.90	1.133	0.171	.000	HS
	First month	Second month	9.37	0.533	0.133	.001	HS
OS	Before mascara use	First month	10.50	0.667	0.146	.000	HS
	Before mascara use	Second month	9.83	1.067	0.172	.000	HS
	First month	Second month	9.43	0.400	0.113	.004	HS
Non- waterproof users							
TBUT [seconds]			**Mean**	**Mean Difference**	**Std. Error**	**P value**	
OD	Before mascara use	First month	10.53	0.567	0.114	0.000	HS
		Second month	9.97	0.933	0.179	0.000	HS
	First month	Second month	9.60	0.367	0.112	0.008	HS
OS	Before mascara use	First month	10.50	0.400	0.123	0.009	HS
		Second month	10.10	0.867	0.196	0.000	HS
	First month	Second month	9.63	0.467	0.164	0.024	sig

OD = Oculus dexter [right eye]; OS = Oculus sinister [left eye] eyelid disease during mascara use in both waterproof and non-waterproof users. Q-3 (100%-no) which states that the subjects have not used any ocular medication/antibiotics during mascara use in both waterproof and non-waterproof users. Q-4 (76.7%-yes and 23.3% no) among waterproof users and (3.3%-yes and 96.7%-no) among non-waterproof users, which states that there was a greater loss of eyelashes among waterproof users when compared to that of non-waterproof users. Q-5 (73.3%-yes and 26.7%-no) among waterproof users and (30%-yes and 70%-no) among non-waterproof users, which shows the burning and itching of eye during a given period of mascara use. Q-6 (100%-no) in both waterproof and non-waterproof users which states that there was no mucous or watery discharge from the ocular surface after mascara use. Q-7 (100%-no) in both waterproof and non-waterproof users which states that there were no conjunctival infections or eyelid redness during mascara use. Q-8 (20%-yes and 80%-no) among waterproof users and (3.3%-yes and 96.7%-no) which states that there was kind of pain or irritation on the eyelid due to constant use. Q-9 (100%-no) in both waterproof and non-waterproof users which states that the subjects did not undergo any trauma on eyelid. Q-10 (3.3%-yes and 96.7% no) among waterproof and (96.7%-yes and 3.3%-no) among non-waterproof users which shows the comfort using mascara.

DISCUSSION

The skin flora refers to the microorganisms that reside on the skin which are usually non-pathogenic, and either commensal (are not harmful to their host) or mutualistic (offer a benefit) it contains 10^3 cells, routinely harbors about 10^4 bacteria, which may be transient or permanent flora. The facial skin flora found near the area of the eye can invade eye cosmetics and contaminate the cosmetics, which under increased circumstances may cause severe eye infections[18]. This study showed that there were growth patterns of facial skin flora that ranged from 10^1-10^5 bacteria which mainly included Staphylococcus species and Micrococcus in mascara tubes that were examined after constant use. Some cosmetics may adhere to eyelashes near to ciliary margin causing eyelash loss and within the lacrimal system and conjunctiva over constant use which may cause decreased tear stability and decreased tear production.

Lactrica et al.,[19] studied microbial contamination associated with mascara use. In their study, they have taken 40 women with an age group of 18-39 years. They did not exclude contact lens wearers,13 subjects included in their study were contact lens users,1 with rigid contact lens and 12 others with soft contact lens. They have used 2 non-waterproof mascaras 1. Avon mascara brand (non-waterproof), 2. Maybelline mascara brand (non-waterproof). In their study, the sample after a given period of mascara use was stored using BBL Culture Swab Plus transport medium (Becton Dickinson and Company, Sparks, Maryland) and transported to a different place for culturing, in their study out of 40 subjects were able to culture only 33 mascara tubes because 7 failed to reach on the day of sample collection. They used 3 different plates for culturing namely blood agar plate, chocolate agar plate and mannitol agar plates. They did not use catalase, coagulation, or selenite F broth test for confirmation of bacteria. The result of their study showed 2 tubes with *Streptococcus epidermidis* growth, 8 showed *Streptococcus* growth, and 4 showed fungal growth. Microbial growth was found in 36.4% of the subject tubes. Based on growth on selective media, most of the organisms were determined to be *Staphylococcus epidermidis, Streptococcus* species, or fungi. Avon brand (non-waterproof) tube showed 31.3% microbial growth among 5 tubes out of 16, and Maybelline brand (non-waterproof) tube showed 29.4% microbial growth among 5 tubes out of 17. The evidence of growth of the same bacteria was seen in contact lens wearers with an increased ratio of multiple colonies. Three Avon control tubes were cultured and there was growth among 2 tubes contributing 66.7% 3 Maybelline control tubes were used which did not show any growth. From the post-study questionnaire after mascara use, they found that 8 subjects from their study were known to treat ocular surface symptoms like dryness, itching or burning complaints. There was no loss of eyelashes amongst mascara users after 3 months of mascara use. The majority had a comfortable response to mascara use.

In the study, 60 subjects were chosen among 90 subjects examined. Thirty individuals were excluded as per the exclusion criteria. The age group ranged between 18-23 years, 28 subjects were in the age group of 18-19 years which constitutes 46.6%, 30 subjects were in the age group of 20-21 years (50%) and 2 subjects in the age group of 22-23 years (3.33%). Two different plates were used for culturing namely, blood agar plate and chocolate agar plate. The gram staining method was used for identification.

Catalase, coagulation, or selenite F broth tests were used for the confirmation of bacteria. Only the mascara tubes were cultured after 2 months of their use. We did not use any other cosmetics like eye shadows or face creams concerning the Wilson and Ahearn study[20] which they have included oil-based and water-based brands. They examined 3 brands with different preservative formulations. The brands mainly used imidazolidinyl urea or p-hydroxybenzoate and twice the concentration of p-hydroxybenzoate in their preservative formulation. In their study heavy growth of bacteria was seen in 2 of the mascara brands investigated which used imidazolidinyl urea or p-hydroxy benzoate in their preservative formulation. It showed *Staphylococcus epidermidis* and *P. aeruginosa*. A third brand of mascara did not allow for additional microbial growth and had moderate bactericidal activity after 2 inoculations with microbes It contained approximately twice the concentration of p-hydroxy benzoate in which thev have been examined. Abdelaziz et al.,[21] had also done a study on microbial contamination of cosmetics, personal care items in Egypt-eye shadows, mascara, and face creams in which Mascara was more contaminated than eye shadows with *Pseudomonas aeruginosa, Citrobacter freundii*, and *Klebsiella pneumoniae*. There was contamination of eye shadows for 70% and face cream was heavily contaminated than mascaras and eye shadows. In the present study, there is no growth of bacteria amongst waterproof users (ADS). The non-waterproof (Dazzler Eyetex) users did not show growth among 4 mascara tubes (13.3%). Staphylococcus aureus growth among 10 mascara tubes (33.3%), micrococcus growth among 7 mascara tubes (23.3%), and both *Staphylococcus* and *Micrococcus* among 9 mascara tubes (15.0%). 2 control tubes, one (non-waterproof) Eyetex brand and one (waterproof) ADS brand did not show any growth. There was the growth of Gram-negative bacteria because the preservatives used in mascaras are likely to be more effective at killing the most likely causes of microbial infection. This may be the reason that no gram-negative bacteria were found because of the offender of infection. Waterproof mainly contains beeswax in the preservative formulation and non-waterproof mascaras use mainly imidazolidinyl urea or p-hydroxybenzoate as the microbial resistance formulation without wax and aqua, hydrated magnesium aluminum silicate, Copernicia cerifera. we have cultured these preservatives in our study, and we have examined the tear production before mascara use, after 1st and 2nd months of mascara use by shimmer-1 and 2, and TBUT. The Shimmers test-1 and 2 before mascara use, after the first and second months of mascara use showed that, in waterproof users, Shimmer test-2 [with anesthesia] showed a statistically significant difference between before and after the first month of mascara use, also a highly significant difference between before and second month of mascara use[22–24].

In non-waterproof users, Shimmer's test-2 (with anesthesia) showed a highly significant difference between before and after the first month of mascara use and a highly significant difference between before and second month of mascara use, as it showed a statistically significant difference in shimmer-2 between the first month and second month. TBUT showed a highly statistically significant difference between before mascara use and after 1st and 2nd month of mascara use in both waterproof and non-waterproof users. It may be because of constant mascara use for a long period that may adhere to the mucous gland of the conjunctiva or beneath the palpebral conjunctiva and its interference with tear film, meibomian gland orifices and superficial ocular surface when worn proximal to the ciliary margin. It may also be due to water resistance components in waterproof users which makes the lashes dry making it difficult to remove which in turn leads to excessive reflex secretions due to irritation and burning on the ocular surface and the discomfort towards the product in use. From post study questionnaire on waterproof users, there was a severe loss of eyelashes amongst users during the assigned period of mascara use (76.7%) reported of eyelash loses after mascara use, which increased over weeks because of the constant use of mascara for 2 months. All waterproof mascara content has agents [beeswax] that dry the lashes and make them difficult to remove which leads to excessive tugging or rubbing on lashes and eye area, which causes loss of eyelashes and irritation and burning sensation.

The study reported that 73.3% of waterproof users with burning and itching complaints during mascara use and 20% of waterproof users had pain or irritation in eyelids due to constant use 96.7% of waterproof users were uncomfortable using the product. In non-waterproof users, there were only 3.3% of eyelash losses among non-waterproof users because of non-water resistance in nature which does not contain beeswax in in their formulation. The study reported 30% of non-waterproof users with burning and itching complaints during mascara use and there were 3.3% of non-waterproof users had pain or irritation on the eyelid due to constant use because of the proximity of cosmetics to the ocular surface over a long time which may interfere with conjunctiva cornea or tear film leading to complain of burning and irritation, 96.7% waterproof users were comfortable using the product. It will have clinical significance in contact lens wearers to avoid the risk of any surface deposits or complications.

LIMITATIONS OF THE STUDY

The study was carried out among the students of AJIAHS, Mangalore. Only 2 mascara brands were used with alpha and beta hemolysis of bacteria. The study was carried out in a particular season of the year, i.e., the rainy season.

SCOPE OF THE STUDY

- It can be studied in other seasons of the year, other regions, other mascara brands containing different preservatives, and contact lens wearers.
- It helps us to provide awareness regarding dangerous ocular infections which may be caused due to the prolonged use of mascara, and eyelashes loss about waterproof mascara use.
- Dry eye associated with constant mascara use in clinical practice.
- Frequent replacement of mascara tubes may be warranted by this study.

CONCLUSION

The waterproof and non-waterproof mascaras have been used by many women constantly so from the study it can be concluded that both waterproof and non-waterproof are not safe for constant use. Waterproof is not at all recommended for daily use because of the risk of severe eyelash loss as non-waterproof mascaras show increased growth of bacteria if used for a long time which may cause severe eye infections also there is a decrease in tear production as per schimmers-2 and TBUT parameters showing highly significant difference because of constant mascara use among both waterproof and non-waterproof users. Further study is needed to determine if more frequent replacement is indicated.

Conflict of Interest

The authors have no conflicts of interest to declare.

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REFERENCES

1. Abelson MB, McGarr PJ. Update on bacterial conjunctivitis. . Available from: http://www/revophth.com/1998/rpj8ttops.html.
2. Ahearn DG, Wilson LA. Microflora of outer eye and eye area cosmetics. *Developments in Industrial Microbiology*. 1976;17:23–28. Available from: https://www.cabidigitallibrary.org/doi/full/10.5555/19761331897.
3. Silbert JA. Ulcerative bacterial keratitis. In: Anterior Segment Complications of Contact Lens Wear. Butterworth-Heinemann. 2000;p. 225–249. Available from: https://books.google.co.in/books/about/Anterior_Segment_Complications_of_Contac.html?id=JKpsAAAAMAAJ&redir_esc=y.
4. Jeng BH, McLeod SD. Microbial keratitis. *British Journal of Ophthalmology*. 2003;87(7):805–806. Available from: https://doi.org/10.1136/bjo.87.7.805.
5. Fleiszig SMJ, Evans DJ. Contact lens infections; can they ever be eradicated? *Eye Contact Lens*. 2003;29(1 Suppl):S67–S71. Available from: https://doi.org/10.1097/00140068-200301001-00019.
6. Rope BL. Waterproof. *Global Cosmet Industry*. 2001;169(2):40–42. Available from: https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=1113539.
7. Bergfield WF, Belsito DV, Jr JGM, Andersen A. Safety of ingredients used in cosmetics. *Journal of the American Academy of Dermatology*. 2005;52(1):125–132. Available from: https://doi.org/10.1016/j.jaad.2004.07.066.
8. Wilson LA, Julian AJ, Ahearn DG. The survival and growth of microorganisms in mascara during use. *American Journal of Ophthalmology*. 1975;79(4):596–601. Available from: https://doi.org/10.1016/0002-9394(75)90798-9.
9. Rope BL. Conquering contamination. *Global Cosmet Industry*. 2002;170(1):40–43. Available from: https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=13450690.
10. Rope BL. Conquering contamination, Part II. *Global Cosmet Industry*. 2002;170(5):40–44. Available from: https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&lang=en&idt=15349716.
11. Rudometkin NJ, Wessels IF, Hedayi RS, Choe JE, Roeske RE. Culture plate temperature and delayed incubation effect on bacterial recovery. *Cornea*. 2003;22(8):746–753. Available from: https://doi.org/10.1097/00003226-200311000-00007.
12. Safe use of cosmetics. . Available from: http://www.uic.edu/com/eye/LearningAboutVision/EyeFacts/Cosmetics.shtml.
13. Rakow PL. Cosmetic use caveats. *Journal of ophthalmic nursing & technology*. 1988;7(6):224–226. Available from: https://pubmed.ncbi.nlm.nih.gov/3199454/.
14. Mah-Sadorra JH, Yavuz SGA, Najjar DM, Laibson PR, Rapuano CJ, Cohen EJ. Trends in contact lens-related corneal ulcers. *Cornea*. 2005;24(1):51–58. Available from: https://doi.org/10.1097/01.ico.0000138839.29823.57.
15. Schaefer F, Bruttin O, Zografos L, Guex-Crosier Y. Bacterial keratitis: a prospective clinical and microbiological study. *British Journal of Ophthalmology*. 2001;85(7):842–847. Available from: https://doi.org/10.1136/bjo.85.7.842.
16. Capaldi PM. When should I replace my mascara? *Contact Lens Forum*. 1988;13(7):9. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=When+should+I+replace+my+mascara%3F+Contact+Lens+Forum+&btnG=.
17. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *British Journal of Ophthalmology*. 2003;87(7):834–838. Available from: https://doi.org/10.1136/bjo.87.7.834.
18. Wilson LA, Ahearn DG. Pseudomonas-induced corneal ulcers associated with contaminated eye mascaras. *American Journal of Ophthalmology*. 1977;84(1):112–119. Available from: https://doi.org/10.1016/0002-9394(77)90334-8.
19. Pack LD, Wickham MG, Enloe RA, Hill DN. Microbial contamination associated with mascara use. *Optometry - Journal of the American Optometric Association*. 2008;79(10):587–593. Available from: https://doi.org/10.1016/j.optm.2008.02.011.
20. Wilson LA, Kuehne JW, Hall SW, Ahearn DG. Microbial contamination in ocular cosmetics. *American Journal of Ophthalmology*. 1971;71(6):1298–1302. Available from: https://doi.org/10.1016/0002-9394(71)90979-2.
21. Abdelaziz AA, Ashour MS, Hefni H, El-Tayeb OM. Microbial contamination of cosmetics and personal care items in Egypt - eye shadows, mascaras and face creams. *Journal of Clinical Pharmacy and Therapeutics*. 1989;14(1):21–28. Available from: https://doi.org/10.1111/j.1365-2710.1989.tb00217.x.
22. Gefken C. Keeping cosmetics-drugs FDA compliant. *Global Cosmetic Industry*. 2005;173(4):64–65.
23. Guide to inspections of cosmetic product manufacturers. 2005. Available from: http://www.fda.gov.ora/inspect_ref/igs/cosmet.html.
24. Perry B. Cosmetic microbiology. *Microbiology Today*. 2001;28(1):185–187. Available from: https://www.scribd.com/document/229040750/Cosmetic-mirobiologi.