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Evaluation of the Microbial Contamination of some Eye-make up Products before and after Use

Objective: The purpose of this study was to evaluate the microbial contamination of eye make up products during handling of consumers. **Materials and Methods**: One hundred eyes make up products including forty eye shadow samples, thirty five eye mascara samples and twenty five eye liner samples of different manufacturers were purchased. The total microbial counts of the eye make up samples were evaluated using spread plate technique. **Results**: The percentages of bacterial organisms at level more than 100 c.f.u/g or ml were found to be 100%, 75% and 36.4% for eye shadow, eye mascara and eye liner, respectively. The percentage of contamination with fungi was found to be 23.1%, 0.0% and 50% at the same order.Moreover, twenty-six eye shadow samples were contaminated with Staphylococcus aureus, Bacillus megaterium, Staphylococcus epidermidis and Klebsiella pneumoniae. While, 13 samples were contaminated with Aspergillus flavus, Aspergillus niger, Fusarium and Penicillium sp. Twenty samples of Mascara were contaminated with Staphylococcus aureus, Staphylococcus warneri, Staphylococcus epidermidis and six samples were contaminated with Aspergillus niger, Fusarium sp. and Aspergillus flavus. Furthermore, eleven samples of eye liner were contaminated with Bacillus cereus, Staphylococcus aureus and Staphylococcus epidermidis. While, fourteen samples were contaminated with Aspergillus niger.
Conclusion: The consumer handling has significant effects on microbial contamination on eye make up products.

INTRODUCTION

Eye cosmetics are defined as cosmetics intended to make eye more attractive, or in some cases to clean the eye area. Most are safe when used properly. However, there are things to be careful about when using these products such as the risk of infection, the risk of injury from the applicator, and the use of unapproved colour additives {1}. According to Harry {2} eye cosmetics are classified into eye shadows, eye mascara and eye liners. The microbial contamination of personal care products may occur in the process of production, through raw materials, ingredients and handling, or even through its repeated use by the consumers {3}. The ability of microorganisms to grow on some types of cosmetic products is common in industry knowledge {4}. Many cosmetic formulations, if improperly preserved, provide a good medium for the growth of bacteria, yeasts and molds and may constitute a health hazards to the consumers. Microbial contamination of aqueous consumer products was especially by gram-negative bacteria have been a serious problem for some manufactures because contamination is often sporadic and may occur in products that meet USP and/or Cosmetic, Toiletry and Fragrance Association (CTFA) acceptance criteria. The causes of contamination are believed to be lack of attention to good manufacturing, practices resulting in the development of house organisms, inadequate preservative systems and/or inadequate microbiological test methods and microbial limits for finished products {5}.
Manufactures also aim, wherever possible, to develop formulations which are incapable of microbial growth. The level of microbial contamination in a non sterile products such as cosmetic formulations, is made clear in the microbial limit standards which should be maintained in the products during their use, in spite of the inevitable contamination by the users, through the addition of a suitable preservative in the products which guarantee the control of microbial level even before they are marketed. Therefore, the need to control microbial contamination of all products for human use and consumption, which support microbial persistence and/or growth, has been of considerable concern to manufacturers {6, 7}. The present investigation was elucidated to determine and isolate the bioburden of some cosmetic eye make up products (intact and in use) which were purchased from different markets in Egypt.

MATERIAL AND METHODS

1-Microorganisms:
A total of one hundred and thirteen microbial contaminants (seventy seven bacterial isolates and thirty six fungal isolates) were isolated from eye make up products obtained from the market. Bacterial contaminants were isolated, purified, and maintained on nutrient agar while, fungi were maintained on Sabouraud,s agar. All cultures were stored at 4°C and subcultured monthly on the same medium.

2-Eye make up Samples:
A total of one hundred-eye make up samples were collected from the market: 40 eye shadow samples manufactured by 7 different companies, 35 eye mascara samples manufactured by 6 different companies and 25 eye liner samples manufactured by 5 different companies. (Tables I-III).

3-Chemicals:
Peptones, Lab-Lemco were products of oxoid. Yeast extract, beef extract were products of BBL. Agar-Agar, tryptone and peptone were the products of Difco. Other chemicals used in the present study were of the reagent grade.

Microbial evaluations of the tested eye make up samples:
Samples were analyzed as soon as possible after purchase. The samples were kept at room temperature. The samples were not incubated, refrigerated, or freezed before or after analysis. Surfaces of samples containers were disinfected with aqueous mixture of 70% ethanol (v/v) before opening and removing contents in a laminar flow cabinet.

The diluent used was: 0.1% tween-peptone (0.1% w/v peptone water, pH 7, containing 0.1% v/v tween 80), in case of powders (eye shadow) and non-fatty products insoluble in water (mascara and eye liner) for the bacterial and the fungal count {8}.

One ml or g of each eye make up sample was mixed with 9 ml sterile tween-peptone and ten fold serial dilutions were made in the same diluent.

a- Total aerobic bacterial counts:

For bacterial counts, the spread plate technique was used, 0.1 ml was taken from each suitable dilution and spread in duplicate sterile plates containing solidified nutrient agar using a presterilized bent glass rod for each dilution. The medium was let to absorp the inoculum. The inverted plates were incubated at 35±2°C and examined daily up to 72 hours. Then, suitable dilutions were counted.

b- Total fungal counts:

For fungal counts, one ml was taken from each suitable dilution and mixed with Sabouraud's agar in sterile duplicate plates. The contents were allowed to solidify. Then, plates were incubated at 28°C ± 2°C and examined daily up to 7 days. Suitable dilutions were counted.

For bacteria and fungi, the number of microorganisms as CFU in each sample is computed by multiplying the average number of colonies per plate by the reciprocal of the dilution used.

Microbial evaluations of the tested eye make up during (in-use): Eye make up samples that showed no contamination with microorganisms were chosen and used in the present investigation. Samples of (eye shadow, eye mascara and eye liner) were applied as usual by consumers every day up to 28 days. Samples of each eye make up product (0.1 g or ml / 0.9 ml diluent) were withdrawn each week for microbial viable count, as mentioned before. According to the morphological characters of the microbial isolates, they were separated on nutrient agar for bacteria and on Sabouraud's agar for fungi, purified and then kept on slants of the same medium, at 4°C for further investigations.

Identification of the bacterial isolates: All plates were examined and morphologically dissimilar colonial types were cultured onto MacConkey agar and Baird parker media. Gram stains of all morphological dissimilar colonial types obtained in pure cultures were carried out. The identification procedures were carried out.

Identification of the Gram Positive rods: Identification of the Gram positive rods were performed according to the methods described by Cowan et al., {9}.

Identification of the Gram Positive cocci and the Gram-negative rods: The apparatus used for the identification of Gram positive cooci and Gram negative rods was: Automated Microscan, Liederbach, Germany. Dade Behring. The Gram positive cocci were grown on Baird Parker medium and the Gram negative rods were grown on MacConkey agar. The resulted growths were identified using the automated "Microscan" which contains a variety of biochemical tests to identify the Staphylococcus species and the gram negative bacteria.

Identification of the fungal isolates: The fungal isolates grown on sabouraud's agar medium were identified.

RESULTS AND DISCUSSION

In the present study, evaluation of the total microbial count of 100 eye make up samples, from different manufacturing companies (40 eye shadow samples, 35 eye mascara samples and 25 eye liner samples), reveal that the microbial counts differ for different samples of different companies.

The total counts of bacteria were found to range between 0.0 to $5x10^3$, 0.0 to $7x10^2$ and 0.0 to $3x10^2$ for eye shadow, eye liner and eye mascara, respectively. The total counts of fungi were found to range between 0.0 to $1x10^2$, 0.0 to $0.2x10^2$ and 0.0 to $1.3x10^2$ at the same order as shown in **(Tables I-III)**.The results also show that out of the 40 eye shadow samples, 14 samples (35%) were found to be contaminated with bacteria only in the range of 2x102 c.f.u/g to $4x10^3$ c.f.u/g, 1 samples (2.5%) was found to be contaminated with fungi only ($1.0x10^2$ c.f.u/g), 12 (30%) samples were found to be contaminated with bacteria (1.0 x 10^2 – $5x10^3$ c.f.u/g) and fungi ($0.1x10^2$ – $1.0x10^2$). While, 13 samples (32.5%) showed no detectable microbial contamination. Out of the 35 mascara samples, 18 samples (51.4%) were found to be contaminated with bacteria only ($0.3x10^2$ – $5x10^2$ c.f.u/ml), 4 samples (11.4%) were found to be contaminated with fungi only ($0.2x10^2$ c.f.u/ml), 2 samples (5.7%) were found to be contaminated with bacteria ($0.4x10^2$ – $7x10^2$ c.f.u/ml) and fungi ($0.1x10^2$ – $0.2x10^2$) while, 11 samples (31.4%) showed no detectable microbial contamination. On the other hand, out of the 25 eyeliner samples, 2 samples (8%) were found to be contaminated with bacteria only in the range of $0.3x10^2$ to $0.4x10^2$ c.f.u/ml., 5 samples (20%) were found to be contaminated with fungi only, in the range of $1.1x10^2$ to $1.3x10^2$ c.f.u/ml, 9 samples (36%) were found to be contaminated with bacteria ($0.3x10^2$ – $3x10^2$ c.f.u/ml) and fungi ($0.2x10^2$ – $1.0x10^2$) while, 9 samples (36%) showed no detectable microbial contamination **(Table, IV)**.Among the contaminated samples, the percentage of bacterial organisms at level more than 100 c.f.u/g or ml was found to be 100%, 75% and 36.4% for eye shadow, eye mascara and eye liner, respectively. While, the percentage of contamination with fungi at the same level was found to be 23.1%, 0% and 50% for eye shadow, eye mascara and eye liner, respectively **(Table, V)**.These data consistent with the findings of Abdelaziz et al., {10} who examined eye shadow and eye mascara samples and found that mascaras were generally more contaminated than the eye shadows. 23% of the eye shadows were contaminated (more than 100 c.f.u/g), 37% of the eye mascara were contaminated (more than 100 c.f.u/ml). Also, more than 75% of the examined eye shadows contained fewer than 100 c.f.u/g aerobic bacterial counts. On the other hand, 4%, 15% of eye shadow and mascaras, respectively, were heavily contaminated with more than 104 c.f.u/g or ml. Abdelaziz et al., {11} examined Al Kohl "eye make up" and found that on sterility testing, 85% of the unused samples were contaminated with bacteria or fungi. Over 70% of these items contained more than 100 c.f.u/g of bacteria and fungi and among those samples 20% were heavily contaminated of bacteria and fungi.Identifications of the microbial isolates from the tested eye make up samples of each company are illustrated in **Tables VI, VII,** and **VIII**. It is noted that Staphylococcus aureus strain is the most predominant contaminant between most of the eye make up samples. Survey and percentage of the identified microbial isolates **(Table IX)** reveal that 26 eye shadow samples were contaminated with 38 bacterial isolates namely, Staphylococcus aureus (55.3%), Bacillus megaterium (21.1%), Staphylococcus epidermidis (13.2%) and Klebsiella pneumonia (10.5%). While, 13 samples were found to be contaminated with 16 fungal isolates namely Aspergillus flavus (62.5%), Aspergillus niger (18.8%), Fusarium (12.5%) and Penicillium species (6.3%).

Twenty eye mascara samples were contaminated with 23 bacterial isolates namely Staphylococcus aureus (69.6%), Staphylococcus warneri (13%) and Staphylococcus epidermidis (17.4%). Six samples were found to be contaminated with 6 fungal isolates namely, Aspergillus niger (66.7%), Fusarium species (16.7%) and Aspergillus flavus (16.7%). On the other hand, eyeliner (11 samples) were contaminated with 16 bacterial isolates namely, Bacillus cereus (31.3%), Staphylococcus aureus (43.8%) and Staphylococcus epidermidis (25%). While, 14 samples were found to be contaminated with 14 fungal isolates namely, Aspergillus niger (100%). Investigators Abdelaziz et al., {11} found that no contamination with pathogenic microorganisms occurred in eye shadows. While, out of 9 items of a specific brand of mascara, 3 isolates of Pseudomonas aeruginosa, one isolate of Citrobacter freundi and one isolate of Klebsiella pneumonia were indicated.

In addition, seven different species of Bacillus were indicated on eye products. Approximately, 50% of the examined samples contained Bacillus species, Staphylococcus species, Pseudomonas species, Pseudomonas vulgaris and Serratia marcesncens. Some of the detected Staphylococcus species were of the aureus type. The Federal Food Drug and Cosmetic (FD & C, Act) defines cosmetics as articles applied to the human body, for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions. Included in this definition are products such as eye and facial make up preparations. Many cosmetic formulations, if not properly preserved, provide a good medium for the growth of microorganisms and as such may constitute a health hazard to the consumer keeping in mind that a finished product rejection due to the presence of these microorganisms can be costly, remembering that a final product is a chain made of number of links. It makes sense to minimize potential weakness at all links in the chain whose weakness could be the introduction of harmful microorganisms. On the other hand, FDA {12} stated import alerts due to eye mascara with mammary seed oil contaminated with gram-negative rods and eye liner contaminated with Pseudomonas aeruginosa. Cosmetic products are used all over the world although aiming at the same high level of consumer protection, their regulations and requirements are quite different from one part of the globe to another. The warm and rather humid climatic conditions that prevail in most tropical countries would tend to support the survival and growth of many microorganisms. In a situation, whereby a nutritionally rich cosmetic product is severely contaminated, rapid growth and multiplication would be expected. This could lead to biodegradation of the product and hence the risk of infection to consumers of the product. Product contamination may arise from raw materials or water used in formulation or accidentally, during in-use {13}. From the moment the cosmetic product is opened until the consumer discards it, it is subjected to constant and variable microbial contamination from the domestic environment and the consumers' hands and body fluids {14}. In the present investigation, the microbial evaluation of the tested eye makeup samples during in-use was performed on the samples that proved to have non detectable microbial contamination, so this study included 13 eye shadow samples, 11 eye mascara samples and 9 eye liner samples (Table, 10). Results reveal that the level of contamination of almost all the studied eye make up samples was found to increase with time and during use except two eyeliner samples, which showed no detectable microbial, contamination. After 28 days. Eye shadow samples were found to be contaminated with Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus albus (up to $7x10^4$ c.f.u), Aspergillus and Fusarium species (up to $8x10^3$ c.f.u). Eye mascara samples were found to be contaminated with Bacillus lichiniformis, Staphylococcus aureus, Bacillus coagulans (up to $0.5x10^4$ c.f.u), Fusarium and Aspergillus species (up to $0.7x10^2$ c.f.u). While, eyeliner samples were contaminated with Bacillus lichiniformis, Staphylococcus aureus (up to $3x10^3$ c.f.u), Fusarium and aspergillus species (up to $6x10^2$ c.f.u).The in-use study performed by Dawson et al.,{15} indicated that the microorganisms recovered from the eye shadow display testers were mainly representative of the normal skin flora and early borne contaminants. Their in-use study resulted in the isolation of Micrococcus species, Cornybacterium species, Staphylococcus epidermidis, Bacillus species, molds and Staphylococcus aureus. While, Gram negative rods, yeasts and Neisseria species were rare contaminants. They stated that most contamination was probably introduced into the cosmetics by the frequent and common use of fingers and multiple use applicators (from tipped swabs or brushes) to sample and spread the different eye shadows onto the eye lid. Applicators were never cleaned or disinfected by personnel and some applicators were worn out from over use.Bacillus species, Staphylococcus species, Pseudomonas species, Pseudomonas vulgaris and Serratia marcescens were recovered from the in-use samples in different percentages and some of the detected Staphylococcus aureus type and one isolate of Pseudomonas aeruginosa {12}. The in-use study was showed that fungal isolates were detected in used eye products {6}. It is clear that aqueous cosmetic products in multiple use containers are subjected to microbial contamination and spoilage unless they are satisfactorily preserved and packaged {16}.

CONCLUSIONS

Eye make up samples were found to be contaminated (intact and in-use) with Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus warneri, Bacillus species, Klebsiella pneumonia, Aspergillus species. Fusarium species and Penicillium species. The major contamination was with Staphylococcus aureus and Staphylococcus epidermidis which were proved to be the main pathogens associated with corneal ulcers and blepharitis. The Eye shadow samples showed a higher bioburden than eye mascara and eyeliner samples. The consumers play an important role in contaminating their eye make up samples during in-use.

Table I: Determination of the total microbial counts and number of microbial isolates of eye shadow samples:

Company	No. S.	Bacteria		Fungi	
		L.C.	No. Is.	L.C.	No. Is.
A	10	$0.0\text{-}8.0\times10^2$	5	$0.0\text{-}0.5\times10^2$	3
B	5	0.0-0.0	--	0.0-0.0	--
C	5	$1.8\times10^3\text{-}4.0\times10^3$	10	0.0-0.0	--
D	5	$0.0 – 5\times10^3$	8	$0.0\text{-}1.0\times10^2$	5
E	5	$0.0\text{-}6.0\times10^2$	4	$0.0\text{-}1.0\times10^2$	4
F	5	$1.0\times10^2\text{-}7.0\times10^2$	5	$0.0\text{-}0.4\times10^2$	2
G	5	$0.0\text{-}6.0\times10^2$	6	$0.0\text{-}0.5\times10^2$	2
Total :7	40	--	38	--	16

No.S.: Number of samples L.C. : Level of contamination No. Is: Number of isolates

Table II: Determination of the total microbial counts and number of microbial isolates of eye mascara samples.

Company	**No. S.**	**Bacteria**		**Fungi**	
		L.C.	**No. Is.**	**L.C**	**No. Is.**
A	5	$0.5\times10^2\text{-}7.0\times10^2$	5	$0.0\text{-}0.1\times10^2$	1
B	5	0.0-0.0	--	0.0-0.0	--
H	5	$0.4\times10^2\text{-}3.0\times10^2$	5	0.0-0.0	--
I	5	$0.0\text{-}3.0\times10^2$	6	$0.0\text{-}0.2\times10^2$	1
J	10	$0.0\text{-}3.0\times10^2$	3	$0.0\text{-}0.2\times10^2$	2
K	5	$0.0\text{-}5.0\times10^2$	4	$0.0\text{-}0.2\times10^2$	2
Total : 6	35	--	23	--	6

No.S. : Number of samples L.C. : Level of contamination No. Is: Number of isolates

Table III: Determination of the total microbial counts and microbial isolates of eye liner samples.

Company	No. S.	Bacteria		Fungi	
		L.C.	No. Is.	L.C	No. Is.
A	5	$0.3\times10^2\text{-}0.5\times10^2$	10	$0.3\times10^2\text{-}1.0\times10^2$	5
B	5	0.0-0.0	--	0.0-0.0	--
H	5	$0.0 - 3.0\times10^2$	4	$0.0 - 0.3\times10^2$	4
I	5	$0.0\text{-}0.4\times10^2$	2	0.0-0.0	--
K	5	0.0-0.0	--	$1.1\times10^2\text{-}1.3\times10^2$	5
Total: 5	25	--	16	--	14

No.S. : Number of samples L.C. : Level of contamination No. Is : Number of isolates

Table IV: Survey on the microbial contamination of the tested eye make up samples of different companies.

Condition	**Eye shadow**	**Eye mascara**	**Eye liner**
No. of companies	7	6	5
No. of samples	40	35	25
No. of C.S. with bacteria only **(%)** Level of contamination with bacteria	14 (35%) $2\times10^2\text{-}4\times10^3$ (c.f.u/g)	18 (51.4%) $0.3\times10^2\text{-}5\times10^2$	2 (8%) $0.3\times10^2\text{-}0.4\times10^2$ (c.f.u/g)
No. of C.S. with fungi only (%) Level of contamination with fungi	1 (2.5%) 0.1×10^2 (c.f.u/g)	4 (11.4%) 0.2×10^2 (c.f.u/ml)	5 (20%) $1.1\times10^2\text{-}1.3\times10^2$ (c.f.u/g)
(%) No. of C.S. with bacteria and fungi (B & F.)	12 (30%) $1.0\times10^2\text{-}5\times10^3$ (B.) $1.0\times10^2\text{-}1.0\times10^2$ (B.)	2 (5.7%) $0.4\times10^2\text{-}7.0\times10^2$ (B.) $0.1\times10^2\text{-}0.2\times10^2$ (F.)	9 (36%) $0.3\times10^2\text{-}3.0\times10^2$ (B.) $0.2\times10^2\text{-}1.0\times10^2$ (F.)
No. of N.C.S. **(%)**	13 (32.5%)	11 (31.4%)	9 (36%)

C.S. : contaminated sample N.C.S. : Non-contaminated samples

Table V: Percentage of the microbial contamination in the tested eye make up samples.

Eye make up	No. of samples	bacteria			Fungi (molds)		
		No. of Cont. * samples	No. of samples count	No. of samples count < 100 (%)	No. of Cont. * samples	No. of samples (%) count	No. of samples count < 100 (%)
Eye shadow	40	26	26 (100)	-- (0.0)	13	3 (23.1)	10 (76.9)
Eye mascara	35	20	15 (75)	5 (25)	6	- (0.0)	6 (100)
Eye liner	25	11	4 (36.4)	7 (36.6)	14	7 (50)	7 (50)

* No. of contaminated samples

Table VI: Identification of the microbial contaminants of eye shadow samples.

company	No. C.S.	Microbial contaminants			
		No. B.S.	Bacteria	No F. S.	Fungi
A	5	5	*Staphylococcus aureus*	3	*Aspergillus flavus*
C	5	5 5	*Bacillus megaterium* *Staphylococcus aureus*	-	--
D	4	4 4	*Klebsiella pneumonia* *Staphylococcus aureus*	2 2 1	*Aspergillus niger* *Aspergillus flavus* *Fusarium* species
E	4	4	*Staphylococcus aureus*	2 1 1	*Aspergillus flavus* *Fusarium* species *Penicillium* species
F	5	5	*Staphylococcus epidermidis*	1 1	*Aspergillus niger* *Aspergillus flavus*
G	3	3 3	*Bacillus megaterium* *Staphylococcus aureus*	2	*Aspergillus flavus*

No. C.S. : number of contaminated samples.No. B.S. : Number of bacterial strains. No. F.S. : Number of fungal strains.

Table VII: Identification of the microbial contaminants of eye mascara samples.

Company	No. C.S.	Microbial contaminants			
		No B.S.	Bacteria	No F. S.	Fungi
A	5	5	*Staphylococcus aureus*	1	*Aspergillus niger*
H	5	5	*Staphylococcus aureus*	--	--
I	4	3 3	*Staphylococcus aureus* *Staphylococcus warneri*	1	*Aspergillus niger*
J	5	3	*Staphylococcus aureus*	1 1	*Aspergillus niger* *Fusarium* species
K	5	4	*Staphylococcus epidermidis*	1 1	*Aspergillus flavus* *Aspergillus niger*

No. C.S. : number of contaminated samples No. B.S. : Number of bacterial strains. No. F.S. : Number of fungal strains**.**

Table VIII: Identification of the microbial contaminants of eye liner samples.

company	No. C.S.	Microbial contaminants			
		No. B.S.	Bacteria	No. F. S.	Fungi
A	5	5 5	*Bacillus cereus* *Staphylococcus aureus*	5	*Aspergillus niger*
H	4	4	*Staphylococcus epidermidis*	4	*Aspergillus niger*
I	2	2	*Staphylococcus aureus*	-	-
K	5	-	-	5	*Aspergillus niger*

No. C.S. : number of contaminated samples. No. B.S. : Number of bacterial strains. No. F.S. : Number of fungal strains.

Table IX: Survey and percentage of the microbial isolates in the tested eye make up samples.

Eye make up		Bacteria					Fungi			
	S.C.B.	Isolates	No. B.C	No. of B.C	%	S.C.F.	Isolated	No. F.C.	No. of F.C.	%
Eye shadow	26	*Staphylococcus aureus*	38	21	55.3	13	*Aspergillus flavus*	16	10	62.5
		Bacillus megateriam		8	21.1		*Aspergillus niger*		3	18.8
		Staphylococcus epidermidis		5	13.2		*Fusarium* species		2	12.5
		Klebsiells pneumonia		4	10.5		*Penicillium* species		1	6.3
Eye mascara	20	*Staphylococcus aureus*	23	16	69.6	6	*Aspergillus niger*	6	4	66.7
		Staphylococcus warneri		3	13		*Fusarium* species		1	16.7
		Staphylococcus epidermidis		4	17.4		*Aspergillus flavus*		1	16.7
Eye liner	11	*Bacillus cereus*	16	5	31.3	14	*Aspergillus niger*	14	14	100
		Staphylococcus aureus		7	43.8					
		Staphylococcus epidemidis		4	25					

S.C.B : Samples contaminated with bacteria. B.C : Bacterial contaminants. S.C.F. : Samples contaminated with fungi with Bacteria F.C. Fungal contaminants

Table X : Survey for the microbial isolates in the tested eye make up samples during handling for 28 days.

make up	Companies	T.S.	Consumer No.	Bacteria			Fungi		
				C.S.	Count range c.f.u/g or ml	Type	C.S.	Count range c.f.u/ g or ml	Type
Eye shadow	A	5	1	5	$5x10^2 – 7x10^4$	*Staphylococcus epidermidis*	5	$0.7x10^2$ $8x10^3$	*Aspergillus* species
	B	5	2	--	--	--	5	$1x10^2$	*Fusarium* species
	E	1	3	1	$1x10^3 – 6x10^3$	*Staphylococcus aureus*	1	$1x10^3 – 8x10^3$	*Aspergillus* species
	G	2	4	2	$1x10^3 – 2.4x10^4$	*Staphylococcus albus*	2	$1x10^2 – 8x10^3$	*Aspergillus* species
Eye mascara	B	5	5	5	$5x10^2$ -$9.5x10^2$	*Bacillus lichiniformis*	5	$1x10^2$	*Fusarium* species
	I	1	6	1	$4x10^2$ -$0.5x10^4$	*Staphylococcus aureus*	1	$2x10^2 – 0.7x10^3$	*Aspergillus* species
	J	5	7	5	$4x10^2 – 7x10^2$	*Bacillus coagulans*	5	$1x10^2 – 4x10^2$	*Fusarium* species
Eye liner	B	5	8	3	$2x10^2 – 1x10^3$	*Bacillus lichiniformis*	2	$4x10^2 – 6x10^2$	*Fusarium* species
	H	1	9	1	$4x10^2 – 3x10^3$	*Staphylococcus aureus*	1	$3x10^2 – 1.3x\ 10^2$	*Aspergillus* species
	I	3	10	--	--	--	3	$2x10^2 – 5x10^2$	*Aspergillus* species

T.S. : Total used samples. C. S : contaminated samples.

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