Hacettepe University Journal of the Faculty of Pharmacy
Volume 30 / Number 1 / January 2010 / pp. 1-16

Microbiological Investigation of Used Cosmetic Samples

Received : 27.02.2009
Revised : 19.01.2010
Accepted : 12.07.2010

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Introduction

Cosmetic was defined in "Cosmetic Law" (24.03.2005 / No. 5324) published by The Ministry of Health of Turkey as "Cosmetics are all the preparations that were prepared to be used for epidermis, nails, hair, lips, genital organs and teeth and mouth mucosa and their only aim is to clean, give odors, change the morphological appearance and/or to regulate the body odors and keep them in good positions" [1].

According to The Federal Food and Drug Cosmetic Act criteria, cosmetic means the articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and articles intended for use as a component of any such articles; except that such term shall not include soap [2].

Contamination of microorganisms in cosmetics may cause spoilage of the product and when pathogenic, they represent a serious health risk for consumers [3].

Most of the cosmetics are not sterile and they are made of non-sterile raw material [4,5,6]. Although cosmetics do not have to be sterile, limit values have been reported according to the type of the cosmetics [5].

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Therefore these preparations should obey Good Manufacturing Practice (GMP) rules in EU and also in Turkey [1]. Unless obeying these rules, any microbial contamination that occurs may cause harmful effect even for preparations and for users' health.

Microorganisms that should not be allowed to be found in cosmetic preparations are; *Staphylococcus aureus, Escherichia coli, Salmonella spp., Candida albicans, Clostridium spp.,* and *Pseudomonas aeruginosa.* Limits of microorganisms that can be found in cosmetic preparations are also mentioned. For example; 500 CFU/g in cosmetics that are used for eye area, 1000 CFU/g in other cosmetics in 1g or 1ml of the preparation (2,6). For this reason in investigating the microbial conditions of the cosmetic preparations 2 points are important; the first one is the aerobic microorganism number in 1g or 1ml of the sample and the second one is the existence of some specific microorganisms such as *S.aureus, P.aeruginosa,* and *C.albicans* [7].

Although cosmetics obey the mentioned rules, to control microbial growth and to stabilize any cosmetic product, some form of preservative needs to be used. Antimicrobial preservatives are substances added to dosage forms to protect them from microbial contamination. However, in many cosmetics no expiry date has been reported and may loose the preservative activity and became a potential risk for microbial contamination. In this study it was aimed to determine the microbial contamination and preservative activity in some used cosmetic samples that have not an expiry date report.

Materials and Method

Cosmetic samples

In the study, 20 eyelashes (EL), 20 lipsticks (LP), 13 foundations (FD) and 20 eye shadows (ES) that were used before were investigated in case of microbial contamination and preservative activity. None of the samples had a reported expiry date.

Media and chemicals

Letheen Broth (LabM), Letheen Agar (LabM), Potato Dextrose Agar (Merck), Eosin Methylene Blue Agar (Merck), Cetrimide Agar (Merck),

Mannitol Salt Agar (Merck), Selenit-F (Merck) and XLD Agar (Merck) were used to grow microorganisms during microbial contamination tests. Ethanol (Merck) was diluted to 70% (v/v) and used for the disinfection of the sample packages and Tween-80 (Merck) was used to disperse insoluble samples.

Microorganisms

Staphylococcus aureus ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 and *Escherichia coli* ATCC 25922 were used in the study for the Microbial Challenge Test.

Colony count and identification

The surfaces of the sample containers were disinfected with aqueous mixture of 70% ethanol (v/v) before opening and removing contents [8].

Eyelashes were not weighed and the inoculations were performed by sterile swabs so in the eyelash samples colonies were not counted [9].

Other samples were aseptically removed and 1g sample was weighed and lipsticks were dispersed in 1ml Tween-80 with glass beads. Then the total mix was mixed with vortex. The total volume was adjusted to 10ml with 8ml Leethen Broth (LB). This suspension was the 10^{-1} dilution and diluted decimally in LB to obtain 10^{-1}-10^{-6} dilution series. 100µl of each dilution was inoculated onto Leethen Agar (LabM) for counting Total Aerobic Bacteria. The inoculum was spreaded with bent glass rod. 100µl of the 10^{-2} diluted suspensions were also added to Potato Dextrose Agar, Eosin Methylene Blue Agar, Cetrimide Agar, Mannitol Salt Agar, Selenit-F and XLD Agar for detection of total fungi, Gram negative bacteria, *Pseudomonas spp.*, *Staphylococcus spp.* and *Salmonella spp.* respectively. PDA plates were incubated at 30±2 °C and observed daily for 7 days. Other plates were incubated for 24 hours at 35±2 °C in aerobic conditions [8].

Plates containing 25-250 colonies were counted and the results were recorded per dilution counted. Average colony counts were multiplied by 10 and then the dilution factor. Results were reported as CFU/g [8].

For the identification of the microorganisms, firstly, Gram staining and microscopic examination was performed. After that biochemical identification tests were done [10].

The Microbial Challenge Test

Microbial challenge test was applied through the method reported by Campana et al. In addition to *Staphylococcus aureus* and *Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* were also included in the study [3]. Samples were placed into sterile containers. Lipsticks were not included in the challenge test because they were dispersed with Tween-80 and the neutralizing activity of Tween-80 might change the activity results. Other samples were inoculated with the standard strains of *S. aureus, P. aeruginosa, E.coli* and *C.albicans* separately. Tryptone soy broth was added onto the samples that contain bacteria and Sabouraud dextrose broth was added onto the samples that contain fungi. The final inoculum of each microorganism was 10^6 CFU/ml.

The samples were well mixed until a homogeneous suspension was determined. Samples were shaken and maintained at room temperature. After a contact time of 0, 3, 7, 14, 21 and 28 days, 1 ml aliquots were removed and placed onto 9 ml of neutralizing medium Leethen broth. Cell viability was determined by the plate count method on TSA and CFU were counted after 24 h incubation at 37 °C. A reduction in the number of each microorganism of 99.9% by 7 days was required in order for the formulation to pass the test [3].

Results and Discussion

In our study, among 73 samples, in 3 eyelashes, 3 eye shadows, 2 lipsticks and 2 foundations of 10 samples microbial contamination was observed. In 5 samples total aerobic bacteria numbers were off the limits. *Salmonella spp.* and *P. aeruginosa* were not observed but *Candida spp.*, *S. aureus* and *E.coli* that are not allowed to be found in cosmetics were determined (Table 1). After the challenge test the preservative activity of all the products was shown to be ineffective because the microbial growth was not limited with a reduction of 99.9% for 7 days.

At time zero, in 34 samples, number of *C.albicans* cells were between 1×10^4-1×10^6. In 19 samples, *C.albicans* growth was not observed so 99.9% reduction was determined in 19 samples at time zero. After 3 days, in all samples growth was determined and in 12 samples, number of *C.albicans* cells were too numerous to count. In other samples, the number of cells were between 3.8×10^5 and 8×10^6 CFU/ml. After 7 days

TABLE I

Samples that showed microbial growth and the isolated microorganisms.

Sample / M.o.	EL1	EL2	EL3	LP1	LP2	ES1	ES2	ES3	FD1	FD2
S. aureus	-	-	+*	-	+	-	-	-	-	-
S. epidermidis	+	-	-	-	-	-	-	-	-	-
Streptococcus spp.	-	-	-	-	-	-	+	-	+	-
Bacillus spp.	-	+	-	-	-	-	+	-	+	-
E.coli	-	-	+	-	-	-	-	-	-	-
Candida spp.	-	-	+	+	-	-	-	-	-	+
Total fungi (CFU/g)	-	-	-	$1\pm0.25\times10^3$	-	-	-	-	-	$4\pm0.17\times10^3$
Total aerobic bacteria (CFU/g)	-	-	-	-	-	$2\pm0.1\times10^3$	$1\pm0.18\times10^3$	$2\pm0.11\times10^3$	$1\pm0.17\times10^3$	$4\pm0.06\times10^3$

* Coagulase positive

EL: Eyelash, LP: Lipstick, ES: Eyeshadow, FD: Foundation

TABLE II

Number of *Candida albicans* cells at time zero (CFU/ml) after Microbial Challenge Test.

	CFU/ml		CFU/ml		CFU/ml		CFU/ml		CFU/ml
EL1	$1.4\pm0.11\times10^5$	EL11	$5\pm0.04\times10^4$	FD1	0	FD12	$1.4\pm0.03\times10^5$	ES10	$2\pm0.2\times10^4$
EL2	$1\pm0.21\times10^4$	EL12	0	FD2	0	FD13	$6\pm0.47\times10^4$	ES11	$2\pm0.28\times10^4$
EL3	0	EL13	0	FD3	0	ES1	0	ES12	$2\pm0.23\times10^4$
EL4	0	EL14	0	FD4	0	ES2	0	ES13	$8\pm1.3\times10^4$
EL5	$2.5\pm0.46\times10^5$	EL15	0	FD5	0	ES3	$1\pm0.05\times10^4$	ES14	$2\pm0.24\times10^4$
EL6	0	EL16	$1\pm0.16\times10^6$	FD6	$3\pm0.24\times10^4$	ES4	0	ES15	$1.2\pm0.05\times10^5$
EL7	$5.5\pm1.5\times10^5$	EL17	0	FD7	$1.2\pm0.16\times10^5$	ES5	$4\pm0.5\times10^4$	ES16	$1.2\pm0.08\times10^5$
EL8	$7\pm1.18\times10^4$	EL18	$8\pm2.73\times10^4$	FD8	0	ES6	0	ES17	$1.6\pm0.1\times10^5$
EL9	0	EL19	$8\pm0.35\times10^4$	FD9	$2.4\pm0.08\times10^5$	ES7	$2\pm0.2\times10^4$	ES18	$1.4\pm0.2\times10^5$
EL10	$7\pm1.4\times10^4$	EL20	$1.6\pm0.1\times10^5$	FD10	$4\pm1\times10^4$	ES8	$4\pm0.12\times10^4$	ES19	$2\pm0.05\times10^4$
				FD11	$1.2\pm0.13\times10^5$	ES9	$4\pm0.17\times10^4$	ES20	$4\pm0.2\times10^4$

EL: Eyelash, LP: Lipstick, ES: Eyeshadow, FD: Foundation

TABLE III

Number of *Candida albicans* ATCC 10231 cells after 3 days (CFU/ml) after Microbial Challenge Test.

	CFU/ml		CFU/ml		CFU/ml		CFU/ml		CFU/ml
EL1	tntc	EL11	tntc	FD1	tntc	FD12	tntc	ES10	tntc
EL2	$8\pm0.2\times10^5$	EL12	tntc	FD2	tntc	FD13	tntc	ES11	tntc
EL3	$1.2\pm0.1\times10^6$	EL13	tntc	FD3	tntc	ES1	tntc	ES12	tntc
EL4	$3.8\pm0.2\times10^5$	EL14	tntc	FD4	tntc	ES2	tntc	ES13	tntc
EL5	tntc	EL15	$1.2\pm0.11\times10^6$	FD5	tntc	ES3	tntc	ES14	tntc
EL6	$2\pm0.2\times10^6$	EL16	tntc	FD6	tntc	ES4	$1.5\pm0.1\times10^6$	ES15	tntc
EL7	$1.5\pm0.1\times10^6$	EL17	$6.5\pm0.31\times10^5$	FD7	tntc	ES5	$8\pm1\times10^6$	ES16	tntc
EL8	$1.2\pm0.1\times10^6$	EL18	tntc	FD8	tntc	ES6	tntc	ES17	tntc
EL9	$1.5\pm0.11\times10^6$	EL19	tntc	FD9	tntc	ES7	tntc	ES18	tntc
EL10	$8\pm0.21\times10^5$	EL20	tntc	FD10	tntc	ES8	tntc	ES19	tntc
				FD11	tntc	ES9	tntc	ES20	tntc

EL: Eyelash, LP: Lipstick, ES: Eyeshadow, FD: Foundation

TABLE IV

Number of *Pseudomonas aeruginosa* cells at time zero (CFU/ml) after Microbial Challenge Test.

	CFU/ml		CFU/ml		CFU/ml		CFU/ml		CFU/ml
EL1	$7\pm1.33\times10^4$	EL11	$2\pm0.25\times10^4$	FD1	$1\pm0.15\times10^4$	FD12	$2.4\pm0.33\times10^5$	ES10	$8\pm0.84\times10^4$
EL2	0	EL12	0	FD2	$2.2\pm0.3\times10^5$	FD13	$1.6\pm0.02\times10^6$	ES11	$1.4\pm0.09\times10^5$
EL3	0	EL13	0	FD3	$1\pm0.09\times10^4$	ES1	0	ES12	$1.4\pm0.05\times10^5$
EL4	0	EL14	0	FD4	0	ES2	0	ES13	$1.8\pm0.17\times10^5$
EL5	0	EL15	$1\pm0.23\times10^4$	FD5	0	ES3	$1\pm0.15\times10^4$	ES14	$6\pm0.53\times10^4$
EL6	$1\pm0.35\times10^4$	EL16	$9.5\pm1.15\times10^5$	FD6	$1\pm0.17\times10^4$	ES4	0	ES15	$5.2\pm0.7\times10^6$
EL7	$1.7\pm0.12\times10^5$	EL17	0	FD7	$3.6\pm0.16\times10^6$	ES5	$1.6\pm0.14\times10^5$	ES16	$5.4\pm0.26\times10^6$
EL8	0	EL18	$2.6\pm0.07\times10^6$	FD8	0	ES6	0	ES17	$1.2\pm0.18\times10^6$
EL9	$2\pm0.27\times10^4$	EL19	$1.4\pm0.15\times10^6$	FD9	$4\pm0.9\times10^5$	ES7	$2.4\pm0.26\times10^5$	ES18	$7\pm1.73\times10^5$
EL10	$3\pm0.32\times10^4$	EL20	0	FD10	$1\pm0.06\times10^6$	ES8	$1.6\pm0.1\times10^5$	ES19	0
				FD11	$3.6\pm0.62\times10^5$	ES9	$1\pm0.04\times10^5$	ES20	$6.2\pm0.22\times10^5$

EL: Eyelash, LP: Lipstick, ES: Eyeshadow, FD: Foundation

number of cells were 4 $\times 10^6$, 2.8 $\times 10^6$, 3.2 $\times 10^6$ and 1 $\times 10^7$ CFU/ml in EL2, EL3, EL6 and EL9 numbered samples respectively. Although number of cells reduced in these samples, the reduction was not enough. After 14, 21 and 28 days in all the samples, number of *C.albicans* cells were too numerous to count. As a result preservative activity was determined only in 19 samples at time zero for *C.albicans.*

At time zero, in 17 samples, *Pseudomonas aeruginosa* ATCC 27853 growth was not observed so 99.9% reduction was determined in 17 samples at time zero. However, after 3, 7, 14, 21 and 28 days number of *Pseudomonas aeruginosa* ATCC 27853 cells were too numerous to count in all the samples. As a result preservative activity was determined only in 29 samples at time zero for *Pseudomonas aeruginosa* ATCC 27853.

For *E.coli* ATCC 25922, only in 5 samples no growth was determined. In other samples numbers of cells were between 1×10^4 - 2.1×10^6 CFU/ml at time zero. After 3 days in all the samples, number of *E. coli* ATCC 25922 cells were too numerous to count. After 7, 14, 21 and 28 days in all the samples number of *E. coli* ATCC 25922 cells were too numerous to count. As a result, preservative activity was determined only in 8 samples at time zero.

After 3, 7, 14, 21 and 28 days number of *Staphylococcus aureus* cells were too numerous to count. In 5 samples at time zero growth was not observed.

For all the microorganisms tested, the 99.9% reduction was determined only in some samples at time zero. After the challenge test the preservative activity of all the products should be ineffective with a reduction of 99.9% for 7 days so in the tested samples, preservative activity was not determined.

Contamination of microorganisms in cosmetics may cause spoilage of the product and when pathogenic, they represent a serious health risk for consumers [3].

"Cosmetics are not expected to be totally free of microorganisms when first used or to remain free during consumer use," according to a 1989 FDA report on contamination of makeup counter samples in department stores. Every time one opens a bottle of foundation or case of eye shadow, microorganisms in the air have an opportunity to rush in. But adequately preserved products can kill off enough of them to keep the product safe [4].

TABLE V

Number of E. *coli* ATCC 25922 cells at time zero (CFU/ml) Microbial Challenge Test.

	CFU/ml		CFU/ml		CFU/ml		CFU/ml		CFU/ml
EL1	$1\pm0.13\times10^4$	EL11	$4\pm0.8\times10^4$	FD1	$6\pm0.75\times10^4$	FD12	$1.6\pm0.17\times10^5$	ES10	$2.8\pm0.26\times10^5$
EL2	$4\pm0.21\times10^4$	EL12	$3\pm0.5\times10^4$	FD2	$3\pm0.43\times10^4$	FD13	$1.1\pm0.26\times10^6$	ES11	$2.6\pm0.36\times10^5$
EL3	$4\pm0.7\times10^4$	EL13	$8\pm0.43\times10^4$	FD3	0	ES1	$2\pm0.26\times10^4$	ES12	$3.4\pm0.35\times10^5$
EL4	$4\pm0.8\times10^4$	EL14	$1\pm0.05\times10^4$	FD4	0	ES2	$1\pm0.15\times10^4$	ES13	$1\pm0.12\times10^5$
EL5	$6\pm0.87\times10^4$	EL15	$2\pm0.43\times10^4$	FD5	$3\pm0.95\times10^4$	ES3	$7\pm0.2\times10^4$	ES14	$2.2\pm0.53\times10^5$
EL6	$7\pm0.12\times10^4$	EL16	$5\pm0.16\times10^5$	FD6	$3\pm1.32\times10^4$	ES4	$2.4\pm0.26\times10^5$	ES15	$1\pm0.06\times10^6$
EL7	$4\pm0.46\times10^5$	EL17	$3\pm0.17\times10^4$	FD7	$1.1\pm0.28\times10^6$	ES5	$3\pm0.43\times10^5$	ES16	$2.1\pm0.29\times10^6$
EL8	$2\pm0.26\times10^4$	EL18	$7.6\pm0.85\times10^5$	FD8	0	ES6	0	ES17	$1.2\pm0.26\times10^6$
EL9	$4\pm0.61\times10^4$	EL19	$1.2\pm0.04\times10^6$	FD9	$9.2\pm0.52\times10^5$	ES7	$4\pm0.2\times10^4$	ES18	$7\pm0.43\times10^5$
EL10	$4\pm1.26\times10^4$	EL20	$7\pm1.8\times10^5$	FD10	$1.2\pm0.09\times10^6$	ES8	$1.7\pm5.1\times10^6$	ES19	0
				FD11	$1\pm0.23\times10^6$	ES9	$2.4\pm4.58\times10^5$	ES20	$8.6\pm4.2\times10^5$

EL: Eyelash, LP: Lipstick, ES: Eyeshadow, FD: Foundation

TABLE VI

Number of *Staphylococcus aureus* ATCC 29213 cells at time zero (CFU/ml) Microbial Challenge Test.

	CFU/ml		CFU/ml		CFU/ml		CFU/ml		CFU/ml
EL1	$7\pm0.2\times10^4$	EL11	$4\pm0.56\times10^4$	FD1	$1\pm0.02\times10^5$	FD12	$3.5\pm0.5\times10^6$	ES10	$1\pm0.36\times10^5$
EL2	$5\pm0.87\times10^4$	EL12	$5\pm0.21\times10^4$	FD2	$6\pm0.7\times10^4$	FD13	$5.1\pm0.36\times10^6$	ES11	$5\pm0.69\times10^5$
EL3	$4\pm0.91\times10^4$	EL13	$4\pm0.34\times10^4$	FD3	$5\pm1.05\times10^4$	ES1	$2\pm0.07\times10^4$	ES12	$1.5\pm0.1\times10^5$
EL4	$2\pm0.26\times10^4$	EL14	$4\pm0.2\times10^4$	FD4	$5\pm0.26\times10^4$	ES2	$9\pm0.43\times10^4$	ES13	$3.6\pm0.11\times10^6$
EL5	$3\pm0.28\times10^4$	EL15	$1\pm0.31\times10^4$	FD5	$3\pm0.1\times10^4$	ES3	$7\pm0.13\times10^4$	ES14	0
EL6	0	EL16	$9\pm0.19\times10^5$	FD6	$1\pm0.21\times10^4$	ES4	$1.3\pm0.38\times10^5$	ES15	$5.5\pm0.49\times10^6$
EL7	$4\pm0.24\times10^5$	EL17	$3\pm0.7\times10^4$	FD7	$3.4\pm0.53\times10^6$	ES5	$1.6\pm0.17\times10^5$	ES16	$1.9\pm0.47\times10^6$
EL8	$4\pm0.27\times10^4$	EL18	$4.3\pm0.26\times10^6$	FD8	0	ES6	0	ES17	$2\pm0.09\times10^6$
EL9	$4\pm0.33\times10^4$	EL19	$3\pm0.12\times10^6$	FD9	$2.1\pm0.36\times10^6$	ES7	$4.4\pm0.28\times10^5$	ES18	$3\pm0.46\times10^6$
EL10	$2\pm0.43\times10^4$	EL20	$2\pm0.12\times10^6$	FD10	$2.7\pm0.67\times10^6$	ES8	$1.8\pm0.36\times10^5$	ES19	0
				FD11	$2.6\pm0.19\times10^6$	ES9	$1.1\pm0.05\times10^6$	ES20	$3.9\pm0.56\times10^6$

EL: Eyelash, LP: Lipstick, ES: Eyeshadow, FD: Foundation

To control microbial growth and to stabilize any cosmetic product, some form of preservative needs to be used. However, in many cosmetics no expiry date has been reported and may loose the preservative activity and became a potential risk for microbial contamination. According to FDA data, most cases of contamination are due to manufacturers using poorly designed, ineffective preservative systems and not testing the stability of the preservatives during the product's customary shelf life and under normal use conditions [4].

Therefore, it is important to improve the preservative system in order to inhibit the growth of contaminating microorganisms during manufacturing, storage and use by consumers [3].

There are several studies that have investigated some unused cosmetics products in case of microbial contamination. Altanlar has studied microbiological quality of 81 lipsticks which are unused. In 81 samples; they have found that 34 samples have been found to be contaminated and total aerobic bacteria counts were between 10^4-10^6 CFU/g. In some lipsticks microorganisms such as mold and yeast which are not allowed to be present in cosmetics were determined [11]. Özdemir has investigated the creams that were prepared by Ege University Department of Chemical Engineering in case of microbial contamination [12]. In only one of the samples *Staphylococcus aureus* was isolated and no other pathogen bacteria mold or yeasts were observed [12]. Ergun, has studied with unused shampoo, hand cream, hair tonic and hair cream samples and in 14 samples the total aerobic bacteria was determined off the limits. 3 *P.aeruginosa*, 2 *E.coli*, 2 *S. aureus*, 5 *Bacillus subtilis*, 2 *Enterobacter spp.* were isolated from the samples [13].

Anar has studied 45 unused and 56 used cosmetics samples (shampoos, creams, mascaras and lipsticks) in case of microbial contamination. Frequencies of bacterial and fungal contamination were 53.47% and 35.64% respectively. 22 unused and 18 used cosmetic samples had pathogenic microorganisms. 12% of used cosmetics products contained more than 10^3 CFU/ml org [9]. Campana et al. have studied 91 commercially available cosmetic products in order to verify the degree of possible microbiological contamination during their use by consumers. They have studied the intact product (at the time of purchase), the in-use products (after 14 days of use) and the ending product (post use). In all cases the contamination was found in ending products, while in one case it was

observed in the in-use product. Also in the study, the preservative systems of the two tested products were studied and they have showed long lasting antimicrobial activity [3].

Ravita et al. have studied with post-consumer use cosmetic products in case of microbial contamination. In this study, densities of culturable aerobic microorganisms of used cosmetic products containing global (GPC) and non-global (NPC) was compared. Among the 96 samples, 28 samples did not yield culturable microorganisms. There was no significant difference between GPC and NPC samples [6]. Preservative activity was not detected by a microbial challenge test in the previous study.

Hugbo et al. have studied with ten brands of commercially available cosmetic creams and lotions. After microbial investigations they have determined that all the products were contaminated to varying degrees. They came to the conclusion that the samples that they were tested did not generally meet the standards for microbial limits. In this study the investigators made a microbial challenge test for preservative activity but in the challenge test they have only used *S. aureus* as a bacteria and *Aspergillus* and *Penicillum* as molds. After the challenge test they have concluded that the preservatives did not possibly possess adequate preservative capacity [14].

Zhang et al. has reported a study on hygienic microbe pollution for imported cosmetics during 2003-2006 and determined that 0.27% of the 4764 cosmetic samples have exceeded the maximum limits of "Hygienic Standard for Cosmetic" in China. As a result of their study they have reported that the quality of the imported cosmetics is satisfactory except sea-mud products whose microbes' proportion exceeds the limitation severely [15].

In our study, among 73 samples, in 10 samples microbial contamination was observed. In 5 samples total aerobic bacteria numbers were off the limits. *Salmonella spp.* and *Pseudomonas aeruginosa* were not observed but *Candida spp.*, *Staphylococcus aureus* and *E.coli* that are not allowed to be founding cosmetics were determined.

After the challenge test the preservative activity of all the products was shown to be ineffective because the microbial growth was not limited with a reduction of 99.9%. At time zero, in 29 samples, *C. albicans* growth was not observed so the growth was limited with a reduction

of 99.99% but to say that the preservative is effective this reduction should continue up to 7 days [3] however after 3 days *C. albicans* cell numbers were increased and a reduction was not observed. *In P. aeruginosa*, at time zero, in 26 samples microbial growth was limited with a reduction of 99.9% but after 3 days the *P. aeruginosa* cell numbers were increased. In *E. coli*, at time zero, in 8 samples microbial growth was limited with a reduction of 99.9% but after 3 days the *E.coli* cell numbers were also increased. At time zero, in 8 samples *S. aureus* growth was also limited with a reduction of 99.9% however after 3 days *S. aureus* cell numbers were increased and a reduction was not observed. The samples that showed preservative activity at time zero are generally the same samples for the tested microorganisms. These results indicate that the preservatives in the studied cosmetics samples are not effective to protect the samples from microbial contamination. The low number of contaminated samples despite the inactivity of preservatives in the samples is thought to be because of the consumers hygienic conditions itself because in our study the samples were all used by one consumer for long time periods. The risk of contamination may be even greater with "testers" at retail stores, where a number of people are using the same sample product [16].

Summary

In this study, our aim was to study the cosmetic samples that were used before and that have not an expiry date report, in case of microbial contamination and preservative activity. A total of 73 samples including 20 lipsticks, 20 eye shadows, 13 foundations and 20 eyelashes that were used before were studied. Microbial contamination was studied according to the guidelines of U.S. Food and Drug Administration (FDA) method: "Microbiological Methods for Cosmetics". Among the samples, in 3 eyelashes, 3 eye shadows, 2 lipsticks and 2 foundations of 10 samples microbial contamination were observed. In 5 samples total aerobic bacteria numbers were off the limits. *Salmonella spp.* and *Pseudomonas aeruginosa* were not observed but *Candida spp.*, *Staphylococcus aureus* and *E.coli* that are not allowed to be found in cosmetics were determined.

Preservative activity was investigated with the Challenge Test. After a contact time of 0, 3, 7, 14, 21 and 28 days, cell viability was determined by the plate count method and a reduction in the number of each microorganism of 99.9% by 7 days was required in order for the formulation to pass the test. After the challenge test the preservative activity of all the products was shown to be ineffective because the microbial growth was not limited with a reduction of 99.9%.

Keywords: Microbial contamination, lipsticks, eye shadows, foundations, eyelashes, preservative activity, challenge test

Özet

Kullanılmış Kozmetik Örneklerinin Mikrobiyolojik Yönden Araştırılması

Bu çalışmada, daha önce kullanılan ve son kullanma tarihleri belirtilmemiş olan kozmetiklerin mikrobiyal kontaminasyon ve prezervatif madde aktivitesi yönünden araştırılmasını amaçladık. Çalışmada daha önce kullanılmış 20 adet ruj, 20 adet göz farı, 13 adet fondöten, 20 adet rimel olmak üzere toplam 73 adet örnek kullanıldı. Mikrobiyal kontaminasyon, Gıda ve İlaç Dairesi (FDA)'nin: "Kozmetikler için Mikrobiyal Yöntemler" metodu ile çalışılmıştır. 3 rimel, 3 göz farı, 2 ruj ve 2 fondöten örneği olmak üzere toplam 10 örnekte mikrobiyal kontaminasyon tespit edildi. Örneklerin 5'inde total aerobik bakteri sayısı izin verilen sınırlar dışında bulundu. *Salmonella spp.* ve *Pseudomonas aeruginosa* gözlenmemekle birlikte, kozmetiklerde bulunmaması gereken *Candida spp., Staphylococcus aureus* ve *E.coli* bazı örneklerde saptandı.

Prezervatif madde aktivitesi, Challenge Test ile saptandı. 0, 3, 7, 14, 21 ve 28 gün sürelerde, hücre canlılığı koloni sayma yöntemiyle saptandı ve 7 gün boyunca her mikroorganizmanın sayısında %99.9'lık azalma olması halinde test sonucu olumlu olarak değerlendirildi. Deney sonucunda hiçbir üründe mikroorganizma sayısında %99.9'luk azalma görülmediğinden hiçbir üründe prezervatif aktivitenin yeterli olmadığı sonucuna ulaşılmıştır.

Anahtar kelimeler: Mikrobiyal kontaminasyon, ruj, göz farı, fondöten, rimel, prezervatif aktivitesi, Challenge test

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