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Microbiological Quality Assessment of Some Brands of Cosmetics Eye Preparations Sold in Libyan Markets

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Abstracts: ***Background: Cosmetic products must be safe to use by the consumers. It is also regulated and required the legislation of countries all over the world . Cosmetic products must be free of pathogenic microorganisms (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosae) and the total aerobic microbial count must be low. Objective of the study: the study was aimed at determining the microbiological quality of some brands of cosmetic eye preparations randomly sold from local markets in Libya. Materials & Methods: A total of 99 samples of different brands of cosmetic eye preparations were analyzed. Results & Discussion: Results showed that most of the products were contaminated. Gram positive bacteria such as Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis and Clostridium perferingens were specifically detected. However all tested samples were free from Gram negative bacteria. A total viable bacterial count ranging from 2.0 X 10^2 to 8.0 X 10^4 cfu/g. The moulds isolated from the cosmetic eye preparations include Penicillium spp., Alternaria spp. and Asperigillus. spp. However Candida spp. was completely absent from the samples analyzed. Conclusion: The cosmetic eye preparations of the studied samples showed to be more contaminated with bacteria than fungi. This may be as a result of poor manufacturing practices, poor hygiene, contaminated raw materials or the susceptibility of the ingredients contained in the cosmetic eye preparations. Therefore, good manufacturing practices and hygiene must be carried out by the manufacturers and personnel. Water must be tested continuously for microbial growth and raw materials should be tested before use especially those of natural origin and cosmetic eye preparations should be stored in an aseptic environment to avoid contamination before vending in the markets.***

Keywords: Microbiological quality assessment, Cosmetic eye preparations, Libyan markets.

1. Introduction

According to The Federal Food and Drug Cosmetic Act criteria (2015), 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 [(1)].

Cosmetic eye preparations are liable to microbial contamination either in the course of their preparation, by the personnel, storage environment, during transportation and/or use by the consumers which may lead to their spoilage. This spoilage may lead to alteration in organoleptic properties of these products which may manifest in terms of changes in color, odor and texture as well as biodegradation of active constituents of such products. Contaminating microorganisms in cosmetic eye preparations may cause spoilage of the product and when pathogenic, they represent serious health risk for consumers worldwide [(2)]. In a situation where by 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 [(3)]. The ability of microorganisms to grow and reproduce in cosmetic products has been known for many years. Bacteria and fungi can get to cosmetics and body care products in several ways. A spoiled product may be described as one that has been rendered unfit for use. As pharmaceuticals and cosmetics are consumed by or applied to the user, manifestations of spoilage are essentially subjective, spoiled can be caused by bacteria, yeast or fungi which are all extremely versatile in their metabolic activities [(4)]. Reports of the microbial quality evaluation of cosmetic products have been from temperate countries and often in response to outbreaks of infectious diseases [(3, 5-7)].

2. Objective of this Study

Is to assess the microbial quality of some selected brands of commonly used cosmetic eye preparations with different dates of production in Libyan markets and to recommend the possibility of some health risk to consumers.

3. Materials and Methods

3.1 Sample Collection

A total of 99 samples were representing three different brands of cosmetic eye preparations. Examined samples were selected at random from unbroken containers obtained from different stores located in Libyan markets. All cosmetic items were having manufacture or expiration dates and the batch numbers were recorded.

3.2 Media Used

Nutrient agar, Blood agar and Mannitol salt agar, Peptone water and Tryptone yeast extract agar were used in the isolation and determination of the bacterial load of the sample. Sabouraud dextrose agar was used for the isolation and enumeration of yeasts and molds. The media were reconstituted and sterilized according to the direction of the manufacturer (Oxoid).

3.3 Bacteriological counts of the Cosmetic Eye Preparations

Stock samples of each cosmetic eye preparations were prepared by dispersing 10g of sample into 90ml of 0.1% peptone water. A ten-fold serial dilution was made and aliquots of the last two dilutions were inoculated on Nutrient agar and second and third dilutions in duplicates were inoculated on culture media using the spread plate method. All the plates were incubated at 37^0C for 24-48 hours followed by colony count. Results were expressed as colony forming unit per gram (CFU/g).

3.4 Yeasts and moulds count of the cosmetic eye preparations:

One ml of the last two dilutions mentioned in above preparations were inoculated on Sabouraud dextrose agar plates using spread plate method. The plates were then incubated at 25^0C for 2-3 days. Colonies were counted after three days. Results of colony count was expressed as yeast and mould counts per gram.

3.5 Identification of Bacterial Isolates

All bacterial isolates were identified based on their Gram reaction and biochemical tests as described by U.S.FDA manual online [8].

3.6 Identification of Fungal Isolates

All fungal isolates were identified based on their macroscopic and microscopic appearance with reference to standard manual [9].

4. Results

Microbial contents of different batches and containers of cosmetic eye preparations:

1-Microbial contents of different batches and containers of kohl:

A total of 18 samples representing two brands of Kohl were tested for their total aerobic bacterial, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (1&2).

The results showed that 50% of the tested samples contained 1000-10000 CFU of aerobic bacteria/g. The other 50% are heavily contaminated and contained more than 10,000 CFU/g. *Bacillus subtilis* was the only aerobic bacteria isolated from all samples (100%) of kohl tested. All samples tested gave zero coliform count and were free from mould and yeast contamination.

2-Microbial contents of different batches and containers of eye shadows:

A total of 27 samples representing three different brands of eye shadow preparations (Global, Miss Rose and Soulafa) were tested for their total aerobic bacteria, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (3-5). Out of nine tested samples of Global eye shadow, three were contaminated with *Bacillus subtilis* with total count of less than 1000 CFU/g.; three samples contaminated with *Staphylococcus epidermidis* with total count less than 1000 CFU/g. Only one sample was contaminated with low count of *Alternaria spp.* with total count of 10 CFU/g and another one was contaminated with low count of *Penicillium spp.* with total count of 10 CFU/g.

Out of nine tested samples of Miss Rose eye shadow, six were contaminated with *Bacillus subtilis* with total count of less than 1000 CFU/g.; three samples contaminated with *Staphylococcus epidermidis* with total count less than 1000 CFU/g. Only one sample was contaminated with low count of *Penicillium spp.* with total count of 3 X 10 CFU/g. All tested samples of Soulafa eye shadow were contaminated with *Bacillus subtilis* with total count of 100-1000 CFU/g. No viable yeast or moulds were isolated from all examined samples.

3-Microbial contents of different batches and containers of Mascara:

A total of 18 samples representing two brands of mascara (**Dulhan** and **My Belloine**) were tested for their total aerobic bacteria, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (6 & 7). Two different viable aerobic bacteria were isolate from examined mascara samples. *Bacillus subtilis* and *Aspergillus spp.* were isolated from Dulhan mascara with total viable count ranges from 300 – 2000 CFU/g and 1000 CFU/g respectively. Only one sample was contaminated with *Bacillus subtilis* alone and one sample was contaminated with mixed *Bacillus subtilis* and *Aspergillus spp. Staphylococcus epidermidis* was isolated from (33.3%) My Belloine mascara examined samples with total viable count ranges from 300 – 2000 CFU/g. There was no fungal contamination observed in this brand.

4-Microbial contents of different batches and containers of Eye liner:

A total of 36 samples representing four brands of eye liners (**Dulhan**, **Lella, Kost and Gardenia**) were tested for their total aerobic bacterial, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (8 - 11). The most commonly isolated aerobic bacteria were *Staphylococcus epidermidis* and *Bacillus subtillis* and only the fungus *Penicillium spp*. were detected.

Out of nine samples of Kost eye liner tested samples, only three were contaminated with less than 1000 CFU/g of *Staphylococcus epidermidis* and only one sample was contaminated with less than 100 CFU/g of *Penicillium spp.*

Also this was observed with Gardenia eye liner brand. However out of nine samples of Dulhan eye liner tested samples only two was contaminated with less than 1000 CFU/g of *Bacillus subtillis* . Also this was observed with only one sample of Lella eye liner brand. No fungal contamination detected in those two brands.

All tested samples of Mascara and eye liner brands were free from anaerobic bacterial contamination. Also all tested samples of Global and Miss Rose eye shadow brands were free from anaerobic bacterial contamination. However *Clostridium perfringens* was detected in all tested samples of Soulafa eye shadow brand and in 100% of the tested samples of the different brands of kohl tested at rates of more than 1000 CFU/g.

5-Anaerobic microbial contents of different batches and containers of cosmetic eye preparations:

Table 1: Aerobic microbial contents of different batches and containers of Hind Ka Noor Kohl.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	6.0×10^3	*Bacillus subtilis*	0	-	-
	2	2.0×10^3	*Bacillus subtilis*	0	-	-
	3	7.0×10^3	*Bacillus subtilis*	0	-	-
B	1	1.0×10^4	*Bacillus subtilis*	0	-	-
	2	1.0×10^3	*Bacillus subtilis*	0	-	-
	3	7.0×10^3	*Bacillus subtilis*	0	-	-
C	1	2.0×10^3	*Bacillus subtilis*	0	-	-
	2	2.0×10^3	*Bacillus subtilis*	0	-	-
	3	3.0×10^3	*Bacillus subtilis*	0	-	-

N.B=No coliform detected.

Table 2: Aerobic microbial contents of different batches and containers of Hashmi Kohl.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	8.0×10^4	*Bacillus subtilis*	0	-	-
	2	8.0×10^4	*Bacillus subtilis*	0	-	-
	3	5.0×10^4	*Bacillus subtilis*	0	-	-
B	1	4.0×10^4	*Bacillus subtilis*	0	-	-
	2	6.0×10^4	*Bacillus subtilis*	0	-	-
	3	2.0×10^4	*Bacillus subtilis*	0	-	-
C	1	3.0×10^4	*Bacillus subtilis*	0	-	-
	2	6.0×10^4	*Bacillus subtilis*	0	-	-
	3	6.0×10^4	*Bacillus subtilis*	0	-	-

N.B=No coliform detected.

Table 3: Aerobic microbial contents of different batches and containers of Global eye shadow

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	6.0×10^2	*Bacillus subtilis*	1.0×10	-	*Alternaria spp.*
	2	2.0×10^2	*Bacillus subtilis*	0	-	-
	3	7.0×10^2	*Bacillus subtilis*	0	-	-
B	1	3.0×10^2	*S. epidermidis*	3.0×10	-	*Penicillium spp.*
	2	3.0×10^2	*S. epidermidis*	0	-	-
	3	4.0×10^2	*S. epidermidis*	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

N.B. 1- *S. epidermidis* = *Staphylococcus epidermidis* 2-No coliform detected.

Table 4: Aerobic microbial contents of different batches and containers of Miss Rose eye shadow.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	9.0×10^2	*Bacillus subtilis*	0	-	-
	2	7.0×10^2	*Bacillus subtilis*	0	-	-
	3	7.0×10^2	*Bacillus subtilis*	0	-	-
B	1	5.0×10^2	*S. aureus*	0	-	-
	2	7.0×10^2	*S. aureus*	0	-	-
	3	4.0×10^2	*S. aureus*	0	-	-
C	1	2.0×10^2	*Bacillus subtilis*	3.0×10	-	*Penicillum spp.*
	2	2.0×10^2	*Bacillus subtilis*	0	-	-
	3	4.0×10^2	*Bacillus subtilis*	0	-	-

N.B. 1- *S. aureus* = *Staphylococcus aureus* 2-No coliform detected.

Table 5: Aerobic microbial contents of different batches and containers of Soulafa eye shadow

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	4.0×10^2	*Bacillus subtilis*	0	-	-
	2	4.0×10^2	*Bacillus subtilis*	0	-	-
	3	3.0×10^2	*Bacillus subtilis*	0	-	-
B	1	5.0×10^2	*Bacillus subtilis*	0	-	-
	2	3.0×10^2	*Bacillus subtilis*	0	-	-
	3	3.0×10^2	*Bacillus subtilis*	0	-	-
C	1	1.0×10^3	*Bacillus subtilis*	0	-	-
	2	2.0×10^2	*Bacillus subtilis*	0	-	-
	3	3.0×10^2	*Bacillus subtilis*	0	-	-

N.B=No coliform detected.

Table 6: Aerobic microbial contents of different batches and containers of Dulhan mascara

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	3.0×10^2	*Bacillus subtilis*	1.0×10^3	-	Asperigellus. spp.
	2	2.0×10^3	*Bacillus subtilis*	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

N.B=No coliform detected.

Table 7: Aerobic microbial contents of different batches and containers of My Belloine mascara

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	2.0×10^2	*St. epidermidis*	0	-	-
	2	2.0×10^2	*St. epidermidis*	0	-	-
	3	3.0×10^2	*St. epidermidis*	0	-	-

N.B. 1- *S. epidermidis* = *Staphylococcus epidermidis* 2-No coliform detected.

Table 8: Aerobic microbial contents of different batches and containers of Kost eye liner

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	0	-	**3**.0 X 10	-	*Penicillium spp.*
	2	0	-	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	2.0×10^2	*St. epidermidis*	0	-	-
	2	5.0×10^2	*St. epidermidis*	0	-	-
	3	4.0×10^2	*St. epidermidis*	0	-	-

N.B. 1- *S. epidermidis* = *Staphylococcus epidermidis* 2-No coliform detected.

Table 9: Aerobic microbial contents of different batches and containers of Dulhan eye liner

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	3.0×10^2	*Bacillus subtilis*	0	-	-
	2	2.0×10^3	*Bacillus subtilis*	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

N.B=No coliform detected.

Table 10: Aerobic microbial contents of different batches and containers of Gardinea eye liner

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	0	-	1.0×10^2	-	*Penicillium spp.*
	2	0	-	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	2.0×10^2	*St. epidermidis*	0	-	-
	2	5.0×10^2	*St. epidermidis*	0	-	-
	3	4.0×10^2	*St. epidermidis*	0	-	-

N.B=No coliform detected.

Table 11: Aerobic microbial contents of different batches and containers of Lella eye liner

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	7.0×10^2	*Bacillus subtilis*	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

N.B=No coliform detected.

Table 12: Anaerobic microbial contents of different batches and containers of cosmetic eye preparation

Batch	Serial No.	Anaerobic microbial contents in					
		Soulafa eye shadow brand		Hind Ka Noor Kohl		Hashmi kohl	
		Total count/g.	Isolated M.O	Total count/g.	Isolated M.O	Total count/g.	Isolated M.O
A	1	4.0×10^3	*C. perf.*	4.0×10^3	*C. perf.*	6.0×10^3	*C. perf.*
	2	3.0×10^3	*C. perf.*	4.0×10^3	*C. perf.*	6.0×10^3	*C. perf.*
	3	3.0×10^3	*C. perf.*	3.0×10^3	*C. perf*	5.0×10^3	*C. perf.*
B	1	2.0×10^3	*C. perf.*	5.0×10^3	*C. perf.*	2.0×10^3	*C. perf.*
	2	4.0×10^3	*C. perf.*	5.0×10^3	*C. perf.*	5.0×10^3	*C. perf.*
	3	2.0×10^3	*C. perf.*	6.0×10^3	*C. perf*	4.0×10^3	*C. perf.*
C	1	3.0×10^3	*C. perf.*	4.0×10^3	*C. perf.*	5.0×10^3	*C. perf.*
	2	5.0×10^3	*C. perf.*	2×10^3	*C. perf.*	5.0×10^3	*C. perf.*
	3	3.0×10^3	*C. perf.*	5×10^3	*C. perf*	7.0×10^3	*C. perf.*

N.B: 1-*C. perf.*=*Clostridium perfringens*. 2- No coliform detected.

5. Discussion

Microbial contamination of cosmetic products is a matter of a great importance to the industry and it can become a major cause of both product and economic losses. The need of the microbial quality of cosmetics is well-clarified and well-recognized. Therefore, this study is aimed to evaluate the cosmetic eye preparations in Libyan market, according to their microbial contents. Results of this study reflect the urgent need to reassess our methods to control the microbial contamination of cosmetics eye preparations in the Libyan market. The results showed that many of cosmetic eye preparations tested contaminated with bacteria in varying degrees including Gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Clostridium perfringens* and all tested preparations were free from Gram negative bacteria. The colony counts of all detected

bacteria are ranging from 2.0 X 10^2 to 8.0 X 10^4 CFU/ml. Also the results showed that some of cosmetic eye preparations tested contaminated fungi including *Penicillium spp*., *Aspergillus*, *Alternaria spp*. and *Asperigillus. spp.* The distribution of microbial contamination between different brands of each class of preparations may reflect one or more of several factors including good manufacturing practice of the manufacturer's post-process contamination, inadequate preservation, extended storage by the retailer etc. The frequency of occurrence of bacteria in many of examined sample shows that most samples are contaminated with bacteria. Thereby indicate that cosmetic eye preparations can permit the growth of bacteria. It was also observed that gram positive organisms were the predominant contaminants in the cosmetic eye preparations. *Staphylococcus* aureus, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Clostridium perfringens,* were the most predominant bacteria. This leads to a presumption that cosmetic eye preparations are more susceptible to gram positive bacteria than gram negative bacteria. This result agrees more or less with that reported by other author [(10)].

The frequency of occurrence of fungi in the total sample indicated that some of the examined samples are contaminated with one form of fungi or the other, which include *Penicillium spp*., *Alternaria spp*. and *Asperigillus. spp.* It also showed that the frequency of isolation of fungal contaminants is lower compared with frequency of isolation of bacterial contaminants. However it was noticed that yeasts contamination was absent in the current study. Similar study also reported more of bacterial than fungal contamination [(11)]. In previous study, isolated *Staphylococcus aureus*, *Clostridium tetani, Candida albicans, Bacillus spp., Aspergillus niger, Aspergillus fumigatus, Penicillium spp, Rhizopus oligosporus, Fusarium spp.*[(7)], while in other study isolated *Staphylococcus aureus, Escherichia coli, Enterobacter agglomerans* and *Citrobacter freundii.* Fungal and bacterial contaminants in unused cosmetic powder are common because of the environment in which the preparatios are manufactured, packed and the ingredients themselves [(5)].The isolation of *Staphylococcus aureus* as the most predominant contaminant tallies with the findings of Ashour *et al*. [(5)].

The International Microbiological Standard recommended limit for bacteria contaminants in cosmetic products is 1.0x10^3cfu/g for bacteria, 1.0x 10^2CFU/g for moulds and Zero CFU/g of coliform at the time they reach the consumer [(12)].

It was observed in the present study that both the total bacterial count and the total fungal count of the cosmetic powder values are more or less within the recommended limits.

Isolation of *Bacillus spp*. which is free living in an indictment of raw materials used as well as the conditions prevalent on the environment in which the products were manufactured and packaged.

Nevertheless, isolation of *Staphylococcus spp*. is a function of personal hygiene on the part of the personnel producing the products since skin is the natural habitat of the organism. *Bacillus spp*. and *Staphylococcus spp*. in cosmetic eye preparations causes eye irritation.

Clostridium perfringens was also isolated which agrees with the findings of Ashour *et al*. [(5)]; who also reported the isolation of *Clostridium spp*. in cosmetic preparations. Clostridium tetani also reported in Gel based creams [(13)]. The presence of the organism poses a serious danger to the user because the neurotoxin produce by the organism is lethal to human. A serious tetanus neonatorum outbreak from talcum powders contaminated with *Clostridium tetani* in New Zealand was reported by Brazier *et al*. [(14)]. The organism gain entrance into the body through cuts on the skin thereby causing infection. The organism is an inhabitant of the soil, which may contaminate the main raw material (talc) of the talcum cosmetic powder [(15)].

Generally, the differences in the manufacturing dates of each product studied, had no significant relevance in the microbial quality as all the samples were sporadically contaminated irrespective of their manufacturing dates. The microbial quality as observed in this study could be caused by air contamination, poor manufacturing practice and improper storage.

6. Conclusion

Microbiological safety is one of the most dynamic and critical of cosmetics quality parameters. From this study it was found that microorganisms such as *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* , *Staphylococcus aureus* , *Staphylococcus epidermidis* , *Penicillium spp.* , *Aspergillus fumigatus* and *Candida albicans* were contaminated in mascara, lip pencil and eye pencil in a varying degrees. The cosmetic industry has many compelling reasons to establish and maintain Microbiological quality of its products. As these rarely produced under a sterile conditions, appropriate control of the many factors involved in the microbiology of the products is critical. These factors include raw material quality, hygiene and training of manufacturing personal, establishment of sanitary design and materials, application of validated cleaning and sanitization process design and control, application of general chemical/physical factors including heat, time temperature, pH addition of specific chemical preservation and use of appropriate barrier packaging . All of these factors are effective for the control of microbiological risks in the cosmetic products.

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